Annals

of the

Missouri Botanical Garden

Volume I

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Annals
of the
Missouri Botanical Garden

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Information

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ANNALS

OF THE

MISSOURI BOTANICAL GARDEN

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Annals
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INTRODUCTION

In order to provide for the printing of scientific papers, which formerly constituted a large part of the volume known as the Annual Report, the Board of Trustees has authorized a new journal, to be known as the ANNALS OF THE MISSOURI BOTANICAL GARDEN. The Annals will appear four times a year, in March, May, September, and November, and contain only scientific contributions from members of the staff of the Garden, from the faculty and graduate students of the Henry Shaw School of Botany of Washington University, and from visiting botanists doing all or a part of their work at the Garden. The increase in original contributions available for publication, due to the additions to the staff and the greater number of graduate students, makes it no longer possible to follow the practice of the past and print papers from sources other than the Garden.

The publication of a monthly bulletin by the Missouri Botanical Garden, in which appear promptly the annual reports of the officers of the Board, and of the Director, together with popular accounts of the various activities of the Garden; and the provision for the printing of scientific papers in the Annals, has made it advisable to discontinue the Annual Report, which was published each year from 1890 to 1912. The Twenty-third Annual Report, therefore, marks the close of this series.

The Annals will be maintained upon a strict subscription basis, using it in exchange only when its equivalent can be obtained. Some of the institutions and societies, the publications of which have been received in exchange for the Annual

Report, issue nothing of value to a botanical library and apparently are not interested in botany or related sciences. These have been stricken from the exchange list. On the other hand, the receipt of this number of the Annals is an indication that the Missouri Botanical Garden desires to continue the old exchange arrangement, the new journal being sent four times a year in place of the old Annual Report. Additional exchanges with publishers of journals dealing directly with botany are desired. Upon request, the monthly BULLETIN will be substituted for the Annals as an exchange with those desiring a more popular and general account of the work and scope of the Missouri Botanical Garden.

GEORGE T. MOORE
Director.

THE EFFECT OF SURFACE FILMS AND DUSTS ON THE RATE OF TRANSPIRATION

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The fungicides commonly employed are either in the form of solutions (e. g., ammoniacal copper carbonate), suspensions (lime wash and Bordeaux mixture), and powders (sulphur). The use of spray mixtures or other fungicides has become world wide, and many problems of physiological interest have arisen respecting the effects of these substances on the plants which they are designed to protect. Bordeaux mixture has been under continuous observation for a period of about twenty years, and has proved interesting in both its toxic and other relations. The striking influence of this fungicide upon sound plants has awakened widespread interest, and numerous experiments have been made to determine the nature of the effects. Bordeaux mixture consists essentially of suspension films of copper hydroxid and certain other complex (mostly hydrated), largely insoluble, copper compounds; and when properly sprayed upon plant surfaces from the best nozzles, the particles are of extreme fineness, and there is realized an almost perfect surface film. In spite of the greatest care in preparation and application, it is injurious to certain plants, such as the peach and the plum, and may not be used satisfactorily in such cases for disease control. In recent years it has been shown that the extent of the injury to the apple and other plants may be considerable, and Bordeaux mixture is in such cases being supplanted. In this discussion, however, we may omit any detailed consideration of the toxic effects of this mixture, a phase of the subject which has received much consideration in this country from Bain (2), Crandall (6), Clark

(4), Swingle (21), and others. Moreover, with the exception of incidental references, we wish to deal at this time only with its physiological action in prolonging the vitality of leaves and plants.

During the first years of the use of this spray mixture it was natural that any increased vitality of the sprayed plants would be attributed merely to the action of the fungicide in restraining fungous or insect pests. Indeed, we find no authentic suggestion of any other effect than that mentioned for eight or ten years after the discovery of this fungicide. Since 1892 there have been frequent observations indicating beyond any reasonable doubt that in the absence of all disease-producing organisms there is often prolonged vitality of the sprayed plants as contrasted with the unsprayed. The increased longevity is particularly noticeable in plants like the potato, in which, under normal conditions, the foliage frequently dies in advance of the first killing frost. Nevertheless, lengthened life in leaves of deciduous trees, notably of the apple, has likewise been reported. It is not always possible to state definitely to just what extent any apparent increased vitality is to be attributed to the physiological action of the fungicide rather than to the control of pests, and it must be said that the frequency of the phenomenon and the reliability of the observers alone preclude the possibility of constant errors in this matter.

In practical field experimentation the most significant differences in yield and vitality as a result of spraying with Bordeaux mixture have been evident in the case of the potato, and with this crop it is a matter of common observation both in Europe and America. In recent years the consecutive reports on potato spraying by F. C. Stewart (19) and his associates at Geneva, New York, suggest in a decisive way the probable magnitude of the Bordeaux influence when disease is a minor factor. In general, observers are perhaps liberal in their estimates of the gain from fungous suppression.

It will be pertinent to note a few observations and comments from the reports of the work done at Geneva. In 1904 the increase in yield from spraying potatoes five times was 233 bushels per acre. "Spraying prolonged the life of the plants 25 days. Late blight was the only trouble." In his experiments

of 1906 Stewart notes an increase in yield of 63 bushels per acre due to spraying five times. He remarks: "Late blight, early blight, flea beetles and tip burn were all factors in this experiment, but none of them caused much damage." More striking were the results the following year when an increase in yield of $73\frac{2}{3}$ bushels per acre was obtained from spraying five times. In this case it is reported: "Late blight and rot were wholly absent and early blight appeared only in traces. There was some tip burn and a light attack of flea beetles. Considering the seemingly small amount of damage done by blight and insects, it is remarkable that spraying should have increased the yield so much." In 1909 the increase in yield in spraying six times was $49\frac{3}{4}$ bushels per acre, and the comment upon this result is as follows: "Early blight, late blight and rot were all absent. Some injury from flea beetles was noticeable throughout the season. After September 1 there was considerable tip burn. As late as September 24 the difference between sprayed and unsprayed rows appeared slight. The sprayed rows held most of their foliage until killed by frost on October 14."

The senior author of this report visited the experimental plats which afforded these data in late September, 1911, prior to the killing frosts of October 27, and the contrast between the sprayed and unsprayed rows was pronounced; at the same time there was very little evidence of any disease on the unsprayed plats. Regarding the condition of the plants Stewart says: "There was no late blight whatever, only a very little early blight, and very little flea beetle injury. The unsprayed rows were affected by no disease of any consequence except tip burn, and even of that there was only a moderate amount. As the plants were still partially alive twenty weeks after planting it is clear that they could not have been very much injured by anything. Yet spraying increased the yield at the rate of 93 bushels per acre. Plainly we have here a striking example of the beneficial influence of Bordeaux in the absence of diseases and insect enemies."

Examining the comments of these and of other investigators regarding increased vitality as a result of spraying with Bordeaux, we find that where the condition of the plant is well

defined at the close of the season, or at the time of the first killing frost, the sprayed plants are almost invariably more vigorous. Often, in the practical absence of any disease, sprayed plants may remain healthy until killed by frost, while unsprayed plants may have died from a few days to a few weeks in advance of frost.

Following a recital of notable increases of yield in Connecticut as a result of spraying potatoes with Bordeaux, Clinton (5) expresses the conviction that an explanation must be found in the conservation of water. His statement follows:

"The question naturally comes up, why did the sprayed potatoes give this increased yield over the unsprayed if there was no particular injury caused by the late blight fungus? Some little benefit was no doubt derived from the prevention of the early blight, but this must have been scarcely appreciable because this fungus was not at all conspicuous these years. Again, some very small benefit may have been due to lessening insect attack, since potatoes sprayed with both Bordeaux and Paris green keep off the insects somewhat better than where sprayed only with Paris green. This is especially true as regards the potato flea beetle. But here again the gain was of a very minor kind. Ordinarily botanists have explained this increase as due to some stimulative effect the Bordeaux mixture has on the chlorophyll of the potato leaves in increasing starch production. Personally, the writer believes that the results are largely due to *conservation of moisture in the leaves in dry seasons by clogging up the stomata and water pores with the sediment of the spray.* The reasons for this belief are (1) that the potato leaves, through their numerous stomata and terminal water pores, lose water very easily, and are especially susceptible to what is known as tip burn in dry seasons; (2) that the unsprayed vines uniformly suffered earlier and more severely from tip burn than the sprayed, which were green for about two weeks after the unsprayed were dead; (3) that in 1910, which was a season like the preceding years, except with a little injury from blight at the very end of the season, spraying with 'Sulphocide' and commercial lime-sulphur, sprays with comparatively little sediment, did not prolong the life of the

vines or give increased yield, while spraying with Bordeaux mixture did."

Although this theoretical explanation did not come to our attention until the experimental work reported in this paper was complete, it was, in modified form, the only possible opinion which we felt inclined to advocate, as a clue to the increased longevity caused by Bordeaux, until the contrary evidence yielded by our experiments.

Review of Literature

The experimental work undertaken in the past to determine the nature of the Bordeaux influence (apart from direct injury) has touched mainly upon (1) questions of increased photosynthesis due either to "stimulation" of chloroplastid or chlorophyll development, or to a direct influence upon light quality; (2) changes in the respiratory rate, or surmised effects upon metabolism; and (3) a modification of the normal rate of transpiration. A few observations from the extensive literature with particular reference to its bearing on transpiration may be cited.

Rumm (16) finds that in sprayed grapes the chlorophyll content of the leaves increases and the fruit ripens earlier with a higher sugar percentage. He attributed these phenomena to the higher "assimilatory activity," and in turn relates this to the following observation on transpiration:—that abscised, sprayed twigs remain fresh longer than those unsprayed, from which it is deduced that there is a falling off in transpiration as a result of spraying. Through independent observations made during the same year, Müller-Thurgau (15) and Bayer (3) subscribe to the view that lessened transpiration follows spraying. Moreover, this confirmation of Rumm is obtained by the former through an experiment which also proclaims that the reduction in transpiration as a result of spraying may be as much as forty per cent. Nevertheless, the report referred to is extremely brief and does not indicate clearly the condition of the plants during the period of observation, a matter most important in the final interpretation of the data afforded.

Frank and Krüger (9, 10) reported some rather extensive

quantitative experiments as a result of which they conclude, contrary to Rumm, that transpiration is accelerated by spraying. They state that sprayed leaves are in general more robust, thicker and stiffer. They also report an increased yield in pot experiments from spraying. All these indications, as well as those of Leydheker (13) and others (1, 12) denote differences of yield which are so slight as to be of no fundamental importance in the present consideration. Nevertheless, the transpiration data of Frank and Krüger, as already observed, were obtained by satisfactory methods, and these are of greater interest when taken in conjunction with those of Zucker (22) who confirms their results entirely.

Schander (18) in an extensive paper reports a comparatively small amount of experimental work on transpiration, but in the cases given his results indicate a retardation of water loss after spraying. His experiments with cobalt paper were inconsistent, and twigs of *Taxus baccata* and potted bean plants were then employed, yielding the positive results noted. However, his work embraced very few plants, and the transpiration differences observed are inconsiderable. He suggests that lessened transpiration of sprayed plants is to be expected, since the Bordeaux mixture must exert a shading influence as a result of the exclusion from the leaf of certain injurious rays. He attempts to verify this assumption of partial shading by a study of leaf temperatures, but the experiments in this direction give no positive evidence for his theory. No adequate mention is made of the conditions surrounding these experiments, nor of the precautions observed.

Ewert's (8) experiments tend to substantiate the views of Rumm and Schander; but, unfortunately, the results are not satisfactory for accurate quantitative purposes, since evaporation from the pots was merely checked and not prevented,batting being employed to cover the soil surfaces. His experiments are of particular interest, however, with respect to his graph for comparative respiration in sprayed and unsprayed plants. In the sprayed plants, respiration was found to be distinctly lower than in the unsprayed. It will be noted, however, that this diminished respiration is scarcely in keeping

with the observation of Rumm and others regarding the higher assimilatory activity in sprayed plants.

It is unnecessary in this report to review the considerable literature which has accumulated bearing on the question of increased starch formation as a result of the application of Bordeaux mixture, especially as it is proposed to discuss this phase of the subject in a later paper.

METHODS

As indicated in the title, the experimental work here reported is concerned merely with the transpiration of sprayed and unsprayed leaves or plants. Other effects of sprays and dusts may be communicated in subsequent reports. In general, the methods involved are modifications of customary practices.

The methods used were of two types, the experiments being carried out either by means of (1) leaves in burette potometers connected with side arm flasks, or (2) potted tomato plants.

Potometer Experiments.—After much preliminary experimentation with a view to determining suitable leaves or twigs for potometer work, leaves of the castor bean were selected. Some of the preliminary experiments with other leaves are of interest, however, and will be referred to subsequently. Castor bean (*Ricinus communis*) leaves offer some special advantages, especially (1) large surfaces, (2) resistance towards Bordeaux mixture, and (3) prolonged vitality after abscision.

The burettes were connected with the side arm flasks, as indicated in plate 1, and the flasks completely filled with water. The petioles of the leaves were cemented into the mouths of the flasks by means of "plastolina." If a ring of this plastic substance is placed around the mouth of the flask when the glass is dry and a ball of the same material, larger than the mouth of the flask, is carefully attached around the petiole, then the petiole and plastolina may be plunged into the mouth of the flask and the two masses unite in a manner such as to give a perfectly air-proof, water-tight connection. It has been found desirable, for purposes of safety, to put on a second layer of the plastolina as soon as it is evident that the first permits no leakage. Even with these precautions, considerable diurnal changes in temperature may cause leakage, and it is particu-

larly important that each experiment should be carefully examined prior to making all readings. The water columns in the burettes were so gauged as to eliminate the possibility of forcing water into the leaves. The burettes were employed solely in order to get accurate readings of the water loss from hour to hour without shifting or disturbing the plants by weighing; also rapidly to get data, should it seem necessary, under changing conditions. All of these considerations proved very important, as it was found that a slight shifting of the position of the leaf affected materially the transpiration magnitudes.

For each leaf used it was necessary to get its rate of transpiration in terms of some standard in order that the ratios might be established between certain leaves prior to the addition of the spray to some of them and the ratio between the same leaves after the application. At one time it seemed possible that the revolving table method of standardizing porous cups might be applicable, but on further consideration it was believed that the use of this method in the laboratory, and the subsequent disposition of the plants in the open, would lead to errors of considerable magnitude. For our purpose it was not considered desirable to conduct the whole experiment on the revolving table, but this method will be employed in connection with our further studies. It was found very important to standardize the leaf in a given position and then permit it to remain in that position, as far as possible, throughout the experiment. This method was necessary largely because of the fact that it seemed wise to conduct the experiment in the open, during a considerable interval, at least. Further reference to the arrangement of the plants will be made in the discussion of the experimental work.

Experiments with potted plants.—For the experiments with potted plants tomatoes were used. The pots were dipped in paraffin wax and the same sealing mixture was coated over the surface of the soil. In all the experiments reported there was no leakage in any case from improper sealing. Water was added daily, or twice a day, to supply the loss by transpiration, the addition of water being made by means of a thistle tube fixed in each pot. The bell of the thistle tube was covered with paraffined paper during the entire interval. It was also

found necessary to insert in each pot a small bent tube in order to provide for the changes in air pressure.

The pots were weighed at the beginning and at the close of the experiment, but the condition of the plant and the amount of water entering readily from the thistle tube were found adequate to indicate the daily water requirements. To the total provisional transpiration quantities obtained from a summation of the quantities daily added, the differences in weight between the beginning and the close of the experiment were added or subtracted as required. From five to ten plants were employed with each kind of spray or dust used, and the plants of each lot were so distributed in the greenhouse that an equal number—so far as possible—from every group was subject to exactly the same influences. Moreover, positions in the greenhouse were shifted several times during the observation intervals of from ten days to two weeks. As a result of a large amount of experimental work in the greenhouse it has become apparent that the points just referred to are important. Plants situated nearer the edges of the benches, or those which receive drafts from opening doors or from convection currents, show considerable differences in transpiration rates, and this should be obviated.

The leaves in the potometer work and the potted plants were sprayed or dusted liberally, and in the case of the sprays, in particular, care was taken to cover completely with a fine spray of the material both surfaces of the leaves. The dust applications were made in the late afternoon when the leaf surfaces were less dry, and after dusting the upper surfaces of the leaves the plants were inverted and the lower surfaces equally well treated. The dusts were prepared by grinding to an impalpable powder in a mortar.

The Bordeaux mixture employed was made by the 4-6-50 formula, the weights of ingredients for making small quantities being approximately as follows:

$CuSO_4$	9.6 grams
CaO	14.4 grams
Water	1000 cc.

The weak Bordeaux was one-half the strength of the above. The $Ca(OH)_2$ was prepared by slaking gradually 60 grams of

CaO in 1 liter of water; and the mixture designated $Al(OH)_3$ was prepared by mixing two solutions each of 900 cc., the one containing 26 grams of $AlCl_3$ and the other 30 grams of CaO (slaked as for the Bordeaux mixture). The clay suspension consisted of 90 grams of fine air-dried clay in 1 liter of water. The lime-sulphur employed was the usual 1–25 strength.

EXPERIMENTAL DATA AND DISCUSSION

It will be observed from the brief review of earlier work that the evidence regarding the effect of Bordeaux mixture on the transpiration rate is inconsistent. A majority of the observers adopt the view that the effect of this surface film is to reduce the transpiration. On a priori grounds this view would seem to be logical, since it would indicate a water conservation to which, in dry seasons at least, the plant might respond with increased vitality and yield. Nevertheless, it was believed that the experimental evidence at hand was of insufficient scope to establish this view of it. Contrary to expectations, all of our more important experimental evidence and observations are antagonistic to the a priori assumption as applied to the effects of Bordeaux mixture.

Potometer experiments.—In attempting to secure leaves satisfactory for the work, some incidental observations were made which are of interest. The work was begun during the winter, so that greenhouse-grown plants alone were available. Furthermore, in this work with potometers, Bordeaux mixture alone has been used by us. Testing leaves of squash (*Cucurbita sp.*), *Pelargonium zonata*, and *Phytolacca*, also shoots of potato and *Irescene*, as to their behavior under the conditions required, it was found that of comparable leaves, sprayed and unsprayed, invariably the sprayed leaves were the first to wilt. This might be attributed either to an injurious action of the spray or to a greater water consumption. That the last mentioned is the more probable explanation finds confirmation through a special observation on the potato. Owing apparently to some stoppage of the vascular system, abscised potato shoots are unsuitable for potometer work, wilting in a comparatively short time even when cut under water; and sprayed potato shoots wilt more quickly than unsprayed, thus pointing

to a more rapid water elimination after spraying. Potted potato plants from which the shoots were cut withstood the fungicide satisfactorily.

Leaves of the large elephant's ear (*Caladium sp.*) proved unsatisfactory on account of the excessive "bloom," which interfered with the proper application of the spray. Canna leaves were similarly unfavorable, and leaves of the calla lily wilt soon after abscision.

It has been stated above that the leaves of the castor bean proved most satisfactory in the potometer work. The experiments with these leaves were carried out in the open, except as otherwise noted, during the early fall. The plants were arranged for standardization and for subsequent observation at distances of about ten feet apart on an exposed lawn uniformly sodded. No readings were made until the leaves had become adjusted to the conditions. Observations were made at frequent intervals when the water loss was rapid, in order to maintain the water column at a fairly uniform level, so that many of the data given in the tables which follow represent summations of several successive readings. Three series of potometer experiments were made, each series embracing six leaves, but in one series, accidents to some of the leaves, and the necessity of substituting new ones after the experiment began, resulted in such a shortening of the standardization intervals that it was thought necessary to discard the results, although they were in the same direction as the others obtained.

The data are presented in full in the tables and all available data are used in computing the relations given. The relations may be more conveniently expressed if we first divide the leaves into classes, designated by letters, as follows:

$A-$, three leaves (i. e., the transpiration quantities of these) in the standardization interval before spraying.

$A+$, the same three leaves as in $A-$, but for any interval after spraying.

B, three control leaves (unsprayed throughout) during the standardization interval.

B', the control leaves as in B, after standardization.

The ratio $\frac{A-}{B}=Q$ is to be compared with the ratio $\frac{A+}{B'}=Q'$.

If Q' is greater than Q, then the spraying facilitates transpiration; if less, then the converse is true. If no accidents occurred during the experiments, $\frac{A-}{B}$ would, of course, be a constant quantity, each term referring properly to the summed transpiration quantities for three leaves during the standardization interval. Accidents are unavoidable, however, during the subsequent observations, and whenever these occur it is necessary to compute a new value of Q' for any particular "run" in which the accident occurs. The only consideration then is to have the same leaves (i. e., their summed transpiration quantities) in the ratios before and after standardization. If, for example, it is necessary to use a ratio, Q', of Nos. 1 and 3 to Nos. 2, 4, and 6 after spraying, then the new value of Q (in the standardization interval) for comparison must also be computed with Nos. 1 and 3 against Nos. 2, 4, and 6.

TABLE I

EFFECT OF FILMS OF BORDEAUX MIXTURE ON TRANSPIRATION OF STANDARDIZED CASTOR BEAN LEAVES; DATA FOR DAY PERIODS

No. of leaf	1	2	3	4	5	6	Ratio
Transp. 12:30–2:30 P. M., 1st day before spraying	$A-$ 10.8	B 17.3	$A-$ 28.5	B 45.9	B 33.8	$A-$ 33.6	$\frac{A-}{B}=\frac{72.9}{97.0}$
Transp. 3:12–5:00 P. M., 1st day after spraying	$A+$ 7.6	B' 20.4	$A+$ 23.2	B' 26.2	B' 26.1	$A+$ 39.5	$\frac{A+}{B'}=\frac{70.3}{72.7}$
Relation, sprayed to unsprayed, 1st day	Rate changed from $\frac{72.9}{97.0}$ (= .75) to $\frac{70.3}{72.7}$ (= .97)						
Transp. 8:12–9:48 A.M., 2nd day after spraying	$A+$ 10.2	B' 37.7	$A+$ 63.1	B' 32.3	B' 29.7	$A+$ 67.1	$\frac{A+}{B'}=\frac{140.4}{99.7}$
Relation, sprayed to unsprayed, 2nd day, *a*.	Rate changed from $\frac{72.9}{97.0}$ (= .75) to $\frac{140.4}{99.7}$ (=1.41)						
Transp. 11:16–11:53 A. M., 2nd day after spraying	$A+$ 4.9	B' 5.9	$A+$ 31.5	B' 13.8	B' 7.5	$A+$ 25.3	$\frac{A+}{B'}=\frac{61.7}{27.2}$
Relation, sprayed to unsprayed, 2nd day, *b*.	Rate changed from $\frac{72.9}{97.0}$ (= .75) to $\frac{61.7}{27.2}$ (=2.3)						

TABLE II

EFFECT OF BORDEAUX MIXTURE ON TRANSPIRATION OF STANDARDIZED CASTOR BEAN LEAVES; DATA FOR DAY AND NIGHT PERIODS

No. of leaf.	1	2	3	4	5	6	Ratio
Transp. 4:04–5:25 P. M., 1st day before spr.	*A*− 7.5	*B* 7.6	*A*− 10.9	*B* 11.7	*A*− 7.6	*B* 9.4	
Transp. 8:21–11:17 A. M., 2nd day before spr.	*A*− 20.2	*B* 30.7	*A*− 32.4	*B* 40.9	*A*− 23.3	*B* 17.9	
Total transp. before spr.	*A*− 27.7	*B* 38.3	*A*− 43.3	*B* 52.6	*A*− 29.9	*B* 27.3	$\frac{A-}{B}=\frac{101.9}{118.2}$
Transp. 12:30–4:50 P. M., 1st day after spr.	*A*+ 36.7	*B′* 42.6	*A*+ 62.9	*B′* 50.2	*A*+ 41.0	*B′* 30.2	$\frac{A+}{B'}=\frac{140.6}{123}$
Relation, sprayed to unsprayed, 1st day	Rates changed from $\frac{101.9}{125.2}$ (=.86) to $\frac{140.6}{123}$ (=1.14)						
Transp. 8:56 A. M., to 4:44 P. M., 2nd day aft. spr.	*A*+ 20.7	*B′* ——	*A*+ 28.9	*B′* 18.2	*A*+ 17.0	*B′* 12.1	$\frac{A+}{B'}=\frac{66.6}{30.3}$
Relation, sprayed to unsprayed, 2nd day	Rates changed from $\frac{101.9}{79.9}$ (=1.28) to $\frac{66.6}{30.3}$ (=2.2)						
Transp. 10:27 A. M., to 3:40 P. M., 3rd day aft. spr.	*A*+ 8.4	*B′* ——	*A*+ 11.2	*B′* 4.4	*A*+ 7.8	*B′* 3.5	$\frac{A+}{B'}=\frac{27.4}{7.9}$
Relation, sprayed to unsprayed, 3rd day	Rates changed from $\frac{101.9}{79.9}$ (=1.28) to $\frac{27.4}{7.9}$ (=3.46)						
Transp. 9:58 A. M., to 4:42 P.M., 4th day aft. spr.	*A*+ ——	*B′* ——	*A*+ 22.1	*B′* 14.1	*A*+ 15.3	*B′* 7.9	$\frac{A+}{B'}=\frac{37.4}{22.0}$
* Relation, sprayed to unsprayed, 4th day	Rates changed from $\frac{74.2}{79.9}$ (=.93) to $\frac{37.4}{22.0}$ (=1.7)						
Total transp. after spraying	—— ——	—— ——	*A*+ 125.1	*B′* 86.9	*A*+ 81.1	*B′* 53.7	$\frac{A+}{B'}=\frac{206.2}{140.6}$
Relation, sprayed to unsprayed, totals	Rates of totals changed from $\frac{74.2}{79.9}$ (=.93) to $\frac{206.2}{140.6}$ (=1.47)						
Transp. 5:30 P. M., to 8:21 A. M., 1st night bef. spr.	*A*− 6.9	*B* 6.3	*A*− 9.0	*B* 11.2	*A*− 6.4	*B* ——	$\frac{A-}{B}=\frac{22.3}{17.5}$
Transp. 4:50 P. M., to 8:40 A. M., 1st night aft. spr.	*A*+ 6.9	*B′* 7.8	*A*+ 2.7	*B′* 7.5	*A*+ 6.2	*B′* 3.5	$\frac{A+}{B'}=\frac{15.8}{15.3}$
Relation, sprayed to unsprayed (night)	Rate changed from $\frac{22.3}{17.5}$ (=1.27) to $\frac{15.8}{15.3}$ (=1.03)						
Transp. 3:45 P. M., to 9:30 A. M., 2nd night aft. spr.	*A*+ 20.0	*B′* ——	*A*+ 15.1	*B′* 4.9	*A*+ 5.8	*B′* 4.2	$\frac{A+}{B'}=\frac{40.9}{4.9}$
Relation, sprayed to unsprayed (night)	Rate changed from $\frac{22.3}{11.2}$ (=2.0) to $\frac{40.9}{4.9}$ (=8.34)						

* For this "run" the plants were transferred to a room in the building.

Summarizing the data for the rates in table II, day intervals, we find that $Q:Q'$, in the successive periods, as .86:1.14, as 1.28:2.2, as 1.28:3.46, and as .93:1.7. If we make the ratio before spraying equal in each case, to 1.0, then the value for the periods after spraying in the successive day intervals are respectively 1.33, 1.72, and 1.83. These differences in rate are so marked and consistent as to outweigh all considerations of individual differences, as disclosed by a detailed study of the figures in table II. It will also be noted that the less extensive data from table I are confirmatory; thus $Q:Q'$, in the successive intervals, as .75:.97, as .75:1.41, and as .75:2.3. On the basis of 1.0 for the ratio before spraying, we have for the periods after spraying, respectively, 1.29, 1.88, and 3.07. From the records of the potometer experiments it is obvious that only one conclusion may be drawn, namely, that the rate of transpiration is materially increased after spraying.

Some points relative to environmental conditions, however, require special mention, and certain suggestive results must be left for further experimental study. Attention has been drawn to the fact that, in general, the potometer experiments were conducted in the open, during early October. During the last days of the work, cooler weather and danger from rain made it desirable to transfer the potometers to a room in the building, and the data for the third and fourth days after spraying, table II, were secured under these new conditions. In this room the shades were drawn and every precaution taken to secure uniformity. It will be noted that while the order of results is in the same direction as for the lawn exposure, the ratio is even higher than the average. No "shading action" of the Bordeaux, as postulated by Schander (18), could be considered a factor of importance in this case.

The results in the laboratory suggest, further, that the ratio of sprayed to unsprayed will vary considerably with the conditions. Before removing the potometers to the laboratory, the night temperatures were so low that two night "runs" (including the interval from about 6 P. M. to 8 A. M.) were necessarily excluded on account of leakage. Other night "runs," as shown in the tables, indicate the probability that under certain conditions unfavorable for evaporation, the surface

film may actually effect a diminution in the rate of transpiration, although the transpiration data do not suffice to warrant more, at present, than an impression. In fact, the night "runs" should be considered apart from those of the day, for the latter are much more satisfactory.

Experiments with potted plants.—The experiments with potted tomato plants were divided into two series which were consecutive in time, and different only with respect to the substances applied to the leaves. As far as has been ascertained, this is the first time that tomato plants have been used in such work, but in our experience they are more satisfactory than potatoes. In the first series (table III) 30 plants were used, in lots of 10 each, for the applications of (1) strong Bordeaux mixture, (2) weak Bordeaux, and (3) controls. In the second series (table IV, V) 80 plants were used in 8 lots, and the substances employed as sprays or dusts are noted in the tables. In the second series it is to be noted that there are 3 substances of the nature of films ($Ca(OH)_2$, $Al(OH)_3$, and lime-sulphur), 1 true suspension (clay), and 3 powders (charcoal, $CaCO_3$, and powdered $Al(OH)_3$).

The methods of procedure involved in these experiments have already been outlined. It is necessary to add, however, that the plants used were about 12 inches high and as uniform in size as could be obtained. It was not possible satisfactorily to standardize plants for an experiment extending over several weeks: and it was necessary to rely in part upon numbers, and in part upon a rigidly accurate method of selecting the individual in each lot to eliminate any errors. The method of selection consisted in getting together 8 plants so similar in size and vigor that no choice could be made between them, then distributing these at random to the 8 lots, this being continued until each lot embraced 10 plants.

In each case the experiments extend over 2 periods. At the close of the first period the plants were shifted in position and a second application of the spray mixture or dust was given. With the conclusion of the experiment the green weights of all plants were taken, thus enabling us to determine, in addition to the total transpiration quantities, the amount of transpiration per gram of green substance.

TABLE III

THE EFFECTS OF BORDEAUX MIXTURE ON THE RATE OF TRANSPIRATION; DATA IN GRAMS FOR 30 POTTED TOMATO PLANTS

	1st period Oct. 18 to Nov. 4		
Covering	Strong Bord.	Weak Bord.	Check
Plants nos.	1–10	11–20	21–30
Transpiration quantities	702	681	390
	684	651	555
	665	540	375
	630	585	525
	625	857	395
	710	440	465
	640	585	415
	445	648	365
	645	645	490
	560	....	545
Total	6306	5622	4520
Ave. per plant	630.6	625	452.0

	Second period Nov. 5–15		
Covering	Strong Bord.	Weak Bord.	Check
Plants nos.	1–10	11–20	21–30
Transpiration quantities	571	628	356
	559	549	554
	575	442	368
	574	603	518
	515	720	385
	740	453	420
	514	499	437
	570	534	417
	495	702	439
	546	....	564
Total	5659	5130	4458
Ave. per plant	565.9	570	445.8

TABLE III (*Continued*)

THE EFFECTS OF BORDEAUX MIXTURE ON THE RATE OF TRANSPIRATION; DATA IN GRAMS FOR 30 POTTED TOMATO PLANTS

Green wts. of plants used.

Plants nos.	1–10	11–20	21–30
Green weights in grams	55	80	49
	58	58	63
	51	41	46
	39	51	56
	50	60	39
	67	45	53
	46	40	48
	46	53	40
	39	74	48
	49	..	55
Total	500	502	497
Ave. per plant	50.0	55.8	49.7

TABLE IV

THE EFFECT OF VARIOUS SPRAYS AND DUSTS ON THE RATE OF TRANSPIRATION; DATA IN GRAMS FOR 80 POTTED TOMATO PLANTS. 1ST PERIOD, OCT. 25 TO NOV. 8

Covering	$Ca(OH)_2$	$Al(OH)_3$	Clay	$Al(OH)_3$ pwd.	Charcoal	$CaCO_3$	Lime-sulfur 1–25	Check
Plants nos.	30–39	40–49	50–59	60–69	70–79	80–89	100–109	90–99
Transpiration quantities	431	394	345	416	378	436	508	352
	437	370	333	370	460	353	414	323
	435	386	430	393	374	315	474	461
	411	383	383	383	460	510	354	443
	358	329	347	645	273	375	526	490
	372	377	520	449	467	346	421	309
	314	398	437	365	320	471	352	330
	416	410	560	531	359	361	346	323
	375	517	362	408	386	456	285	343
	485	460	452	412	331	364	402	317
Totals	4034	4024	4169	4372	3808	3987	4082	3691
Ave. per plant	403.4	402.4	416.9	437.2	380.8	398.7	408.2	369.1

TABLE V

THE EFFECTS OF VARIOUS SPRAYS AND DUSTS ON THE RATE OF TRANSPIRATION; DATA IN GRAMS FOR 80 POTTED TOMATO PLANTS. 2ND PERIOD, OCT. 25 TO NOV. 8.

Covering	$Ca(OH)_2$	$Al(OH)_3$	Clay	$Al(OH)_3$ pwd.	Charcoal	$CaCO_3$	Lime-sulfur 1–25	Check
Plants nos.	30–39	40–49	50–59	60–69	70–79	80–89	100–109	90–99
Transpiration quantities	494	812	624	665	573	560	846	590
	463	587	582	601	610	543	641	569
	592	653	693	609	530	553	667	564
	539	587	615	654	744	700	622	701
	604	654	594	754	556	512	680	764
	457	614	579	606	617	552	616	546
	544	665	694	476	569	706	604	496
	647	605	753	704	664	585	478	558
	597	806	579	641	596	620	601	514
	582	810	590	711	562	617	428	601
Totals	5509	6793	6303	6421	6021	5948	6183	5903
Ave.	550.9	679.3	630.3	642.1	602.1	594.8	618.3	590.3
*Transp. per gm.	11.8	12.2	12.3	11.4	13.1	12.0	12.8	12.1

Green wts. of plants 30–109 at close of 2nd period.

Green weights in grams	39	71	46	58	49	50	56	58
	39	43	47	52	43	47	51	47
	49	58	51	45	48	43	42	38
	42	53	49	79	35	53	53	57
	60	56	54	77	35	53	67	53
	43	56	58	47	59	51	40	56
	41	53	52	47	46	57	52	35
	50	55	50	54	48	47	36	46
	60	58	48	47	52	43	49	46
	43	54	59	55	43	51	39	51
Totals	466	557	514	561	458	495	485	487
Ave. per plant	46.6	55.7	51.4	56.1	45.8	49.5	48.5	48.7

* Computed on the basis of green weights at the close of second period.

An examination of the data in the several tables involved in the pot experiments serve to indicate that while there is a certain amount of individual variation in the transpiration quantities of the various plants in any group, general conclusions seem to be warranted. The individual variations in transpiration in the Bordeaux series are in closer accord with the variations in green weight of the plants used than are those in the other series. Taking all factors into consideration, a film of Bordeaux mixture is found to facilitate transpiration. Other films and dusts employed do not seem to affect the rate of transpiration to the same extent.

In a consideration of the results in detail it is to be noted that the Bordeaux series (table III) is not strictly comparable with the other (tables IV, V), since they were not conducted simultaneously. If the transpiration in grams per gram of green weight for the control (Bordeaux series) is represented by 100, then the rate for weak Bordeaux on this basis is 113.2, and the rate for strong Bordeaux is 125.43. The differences are in the same direction, but not so great as those obtained with the potometer experiments. The use of both weak and strong Bordeaux mixture materially strengthens the conclusions to be deduced.

The series which gives the results with other sprays and dusts is not so easily interpreted. The transpiration quantities vary slightly on either side of the control, and no covering gives a negative difference (contrasted with the control) greater than six per cent (this is the case of $Al(OH)_3$), or a positive difference greater than about eight per cent (charcoal).

These slight average differences may be no more than would be explained by the possible experimental error; but it is of interest to perceive that, with the exception of clay, those surface applications which give lower values than the control are those which might diminish the absorption of heat in direct sunshine. The results might then be the resultant of two factors, (1) the direct effect of the surface film or dust on the rate of water loss, and (2) the indirect effect exerted through a modification of the temperature of the leaf.

Accepting as a general conclusion an acceleration of transpiration (specifically in the castor bean and in the tomato)

as a result of an application of a film of Bordeaux mixture, the following questions arise: (1) What is the physical or chemical basis of the increased evaporation from plant surfaces covered with Bordeaux mixture? (2) Is the increased evaporation in any way related to the increased vitality or longevity of sprayed leaves? Neither of these questions may be answered intelligently at present. With respect to the first, we have arranged experiments to determine the effects of Bordeaux on the passage of water vapor through non-living membranes; but the results are thus far conflicting, due possibly to the fact that we have not yet used membranes which are satisfactory analogues of leaves. Experiments in this direction will be reported later. No relation of transpiration to increased longevity can be foretold, although it seems possible that the highest efficiency equilibrium relation of longevity may involve, in certain plants, a relatively high transpiration rate as either a direct or an indirect factor. No answer to the question will be satisfactory until a further study of other effects ("stimulation," increased "assimilatory activity," etc.) of Bordeaux mixture shall have been made.

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Explanation of Plate

PLATE I

Potometer used in the transpiration experiments showing burette connected with the side arm flask, and abscised leaf of *Ricinus* cemented into the mouth with plastolina.

DUGGAR AND COOLEY—TRANSPIRATION

SOME PURE CULTURE METHODS IN THE ALGÆ

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Introduction

Too much confidence has frequently been placed by algologists in their ability to recognize a given species of alga among varying numbers of other species, and in the various forms which it may assume—a fact which has led to much confusion and error, especially among members of the *Protococcales*. While it is now definitely known that in a number of algæ a single species may present markedly dissimilar appearances, either as a result of varying environmental conditions, or because of the presence in the life history of several unlike stages, it is certain that much of the so-called *polymorphism*, or *pleomorphism*, of algæ finds its explanation in inadequate methods of study. It is becoming recognized that for life history studies in the algæ it is necessary to employ cultures free from other species of algæ. Even in cases where this is not, on first thought, necessary, as in the large, filamentous forms, it should be observed, for the possibility of introducing spores or sporelings of closely allied species is by no means excluded in all cases. Gratifying progress has already been made by some algologists, working especially with members of the *Volvocales* and *Protococcales*, and it seems reasonably certain that the originally chaotic condition existing in the latter will be ultimately reduced to complete order by a careful observance of the necessity of working with pure cultures, or at least cultures containing but a single species of alga. In life-history studies where physiological differences between species are to be investigated, it is especially desirable and indeed necessary to employ pure cultures.

Certain species of algæ, especially representatives of the *Chlorophyceæ*, have been much used in physiological investigations—chiefly those concerning themselves with various

phases of nutrition. With the development of a clearer understanding of the activities and life processes of the various micro-organisms, the necessity of working with rigorously pure cultures has become more and more evident. It is now generally appreciated that, in most cases, valid conclusions as to the physiology of a particular organism cannot be drawn with certainty where one or more foreign organisms have been present in the cultures. There can be no doubt that the frequent contamination of cultures of algæ with bacteria, and even with fungi, has, in many cases, detracted markedly from the value of painstaking and otherwise careful physiological investigations. The readiness, however, with which many algæ lend themselves to experimental purposes—on account of their small size and ease of handling and culture—will always make them favored objects of study; and it appears desirable at this time to bring together some of the experiences of the author in the preparation of pure cultures of algæ, with the hope that suggestions may be gained from them by those who desire to obtain such cultures for one purpose or another.

An unfortunate use of the term "pure culture" has come into more or less general use and has frequently led to confusion and ambiguity. As used by many authors, it means simply a culture of a single species of alga not necessarily free from bacteria and fungi. Where the presence of other organisms is not specifically mentioned, it is clear that the above usage of the term may lead to serious misunderstandings. Indeed, it remains for the reader, in many instances, to decide for himself—from the technique employed—whether a culture of an alga free from all other organisms or only from other species of algæ is meant. It is to be hoped, therefore, that the term pure culture shall come to have the same clearly defined meaning when used in connection with the algæ that it has long had in the fungi and bacteria. In the following report the term is used to signify a culture of a single species of alga free from all other organisms.

Historical

Although incidental references to pure culture technique in the algæ are frequently found in the literature, relatively few contributions have appeared which deal extensively with the

subject, or which outline in detail the methods employed. Beyerinck, in 1890 (4, 6), appears to have been the first to succeed in isolating species of algæ in pure culture. Ditch water, boiled with ten per cent gelatin, and cooled, was mixed with a drop of water rich in protococcoid algæ, poured into dishes, and allowed to cool. Numerous minute algal colonies appeared in course of time, and the number of bacterial colonies developing was so small that successful transfers of *Scenedesmus acutus* Meyen and *Chlorella vulgaris* Bey., were made, both organisms being subsequently cultured on a variety of media. In addition, the gonidia of *Physcia parietina* were obtained pure. Small pieces of the lichen thallus, carefully washed, were placed on solid gelatin plates. Those which showed themselves to be free from foreign organisms were transferred to gelatin plates containing malt-extract, the fragment being first torn to bits with needles and then dragged over the sterile surface. In a few days, small colonies of the algal symbiont appeared from which successful transfers were made. In a later paper (5), Beyerinck adds *Stichococcus major* and a second species of *Chlorella* to the list of algæ previously cultured in a state of purity, the technique, in general, being the same.

Miquel (16) was the first to isolate a diatom in pure culture. Subsequently, Richter (20, 21) isolated *Nitzschia Palea* (Kütz.) W. Sm., and *Navicula minuscula* Grun., by the use of synthetic agar plates. Attention is called by this author to the importance of using agar which has previously been washed to free it from soluble impurities. A mixture of diatoms and other algæ was placed on the surface of washed agar plates, and from the impure diatom colonies which developed transfers were made to other plates until at length pure cultures were obtained.

In his isolations of certain protozoa in pure culture, Ogata (18) also obtained *Polytoma uvella*. While his method seems unnecessarily complex, it is of interest here. Sterile capillary tubes were filled in part with a column of sterile water, and subsequently a column containing the organisms was added below, care being taken not to separate the two by air. Both ends of the tube were then sealed. After sufficient time had elapsed for the movement of the motile *Polytoma* cells from the lower column into the upper sterile one, the tube was broken in

the region of the upper column. The lower portion was discarded, and the upper one was sealed, subsequently transferred to a sterile medium, and broken to permit the organisms, free from contaminations, to enter the medium and begin their development.

By the gelatin plate method, Krüger (13) prepared pure cultures of two new organisms—*Chlorella protothecoides* and *Chlorothecium saccharophilum*—obtained from the exudation of *Populus alba*. Tischutkin (23) lists representatives from about eighteen genera of algæ—including diatoms, green, and blue-green forms—as having been obtained in pure culture by the agar plate method. After three or four successive dilutions in liquid one per cent agar, the organisms were plated in Petri dishes. The filamentous forms he washed in sterile water, cut into short segments, and transferred to the liquid medium. The methods given by Ward (24) include plating in agar and silicic acid jelly, though as a whole the methods are applicable for the separation of algal species rather than for their isolation in pure culture. This is especially true of the plaster of Paris, and precipitated calcium carbonate methods. Gonidia from *Xanthoria parietina*, and *Gasparinia murorum* (Hoffm.) Tornab., together with *Pleurococcus vulgaris* and *Scenedesmus caudatus* were obtained in pure culture by Artari (1). Chodat and Goldflus (8), by the use of pieces of sterilized unglazed porcelain in contact with a mineral nutrient solution, claim to have isolated a species of *Nostoc* in pure culture. The procedure was a simple one, consisting in repeated transfers to fresh sterile plates until a pure culture was at length obtained.

Several years later Chodat and Grintzesco (9) reported that by essentially the same method, *Oocystis elliptica*, *Dictyosphærium pulchellum*, *Kirchneriella lunaris*, *Rhaphidium polymorphum*, *Pediastrum tetras*, *Scenedesmus acutus*, *Pleurococcus vulgaris*, *Hæmatococcus lacustris*, and *Chlorella vulgaris* had been obtained in pure culture. In cases where the number of algal individuals is small, but the bacteria and fungi relatively abundant, the authors point out the desirability of first increasing the number of the former by introducing the mixture into a mineral nutrient solution favorable for the growth of the algæ but not so for the fungi. Where filamentous forms are

concerned, the authors state that it is necessary to begin with the zoöspore, as a pure culture from filaments is extremely difficult to obtain. My own experience does not bear out this statement in all cases as it was found that especially among the *Ulotrichales* pure colonies were regularly and easily obtained from filaments.

Artari in 1902 (2) reports the isolation of *Chlorococcum infusionum* and *Scenedesmus caudatus* in pure culture. Chick (7) attempted to isolate *Chlorella pyrenoidosa* through the use of sterilizing agents such as hydrogen peroxide and sunlight. These trials, however, did not prove successful, as the alga failed to show a resistance sufficiently greater than that of the bacteria to make possible a successful separation. The isolation was finally attained by placing a few drops of water containing the organism on a sterile synthetic agar plate, and spreading the same over the surface with a brush. The same brush was used to distribute sterile water drops over the surface of other plates, no additional algal material being added. From the later dilutions pure colonies were obtained. Frank (10) was unable to obtain pure cultures of *Chlamydomonas tingens* by the agar plate method.

Jacobsen (11) reports the isolation of *Chlorogonium* and *Polytoma* in pure culture. This author made use of an interesting method of separation of algal species based on their different degrees of resistance to drying. Discs of filter paper, on which drops of water containing *Spondylomorum* and *Chlamydomonas variabilis* had been placed, were dried in an incubator at 28°C. After twenty-four hours, the discs were placed in a suitable medium, but only the *Chlamydomonas* species developed, *Spondylomorum* having been killed. *Chlorogonium euchlorum* and *Polytoma uvella* also showed themselves very sensitive to drying, whereas *Chlamydomonas* usually survived the desiccation. Old cultures of *Chlorogonium euchlorum* proved to be very resistant owing to the presence of zygospores which had been formed by the conjugation of gametes.

While reference might be made to a number of other investigations which deal in an incidental way with pure culture technique, it is believed that those given will serve to indicate, in a general way, the present status of the subject. (For further

information the reader is referred to Moore (17), Richter (21), Küster (14), and others.) It is apparent that the large majority of forms isolated in pure culture belong to the *Protococcales*. Only a few of the filamentous forms, several diatoms, and but one or two species of the blue-green algæ have thus far yielded to pure culture technique.

Pure Culture Technique

General

Algæ, generally speaking, are provided with a more or less highly developed exterior mucilaginous investment which may be either a distinct, separable sheath, as in many of the *Cyanophyceæ*, or merely a gelatinization resulting either from a modification of the external portion of the membrane, or from an internal secretion, as in some of the desmids. In general, also, algæ are slow growing as compared with many fungi. In these two characteristics most of the difficulties encountered in pure culture technique among the algæ find their explanation.

Among the fungi, spores with non-gelatinous walls are readily obtainable in a majority of the forms, and usually in great abundance. When such spores are plated in the way ordinarily employed in bacteriological technique, a large number of colonies free from bacteria are usually obtained. Among the algæ, however, such non-gelatinous, resistant spores are, if produced at all, generally present only in small quantities. When vegetative algal cells are plated on a suitable medium, algal colonies will often be obtained, but they usually form the nucleus of a larger bacterial colony which has developed from the bacteria adhering to the gelatinous surface of the algal cell. Among those fungi in which spores are not readily obtained, an isolation in pure culture may frequently be effected by allowing the fungus to grow on a suitable medium until the hyphæ have outstripped the bacteria in their growth, at which time pure mycelial transfers may be made from the terminal portions. If, however, a like procedure is attempted with the algæ it will usually be found that the bacteria adhere tenaciously to the surface of the growing filaments and are carried

along by the lengthening filaments. Except in rare cases, nothing is to be gained by this procedure in the algæ. The task of isolating pure cultures of algæ, therefore, becomes an individual problem for almost every species as it necessitates at once the determination of the period in the life history of any form at which the cells are free from bacteria or at which time the bacteria can be removed by one means or another. Having found a stage in which the alga is bacteria-free, it is of importance next to be able to bring about this stage more or less at will in order that the alga may be utilized when available. To obtain the above preliminary information, nothing is more serviceable than the usual plating method on a suitable medium.

The Medium.—The requirements of a suitable solid medium for algal isolating purposes are, that it remain liquid down to a temperature at which delicate algal cells are not injured; that it be suitable for the growth of algæ, and as unfavorable as possible for the growth of bacteria and fungi. For this purpose nothing was found so serviceable as the following, the mineral ingredients being in the proportions recommended by Moore (17):

Agar	10.0 grams
$NH_4\ NO_3$	0.5 gram
$MgSO_4.\ 7H_2O$	0.2 gram
K_2HPO_4	0.2 gram
$CaCl_2$	0.1 gram
$FeSO_4$	trace
Dist. H_2O	1000 cc.

The agar should be carefully washed, first in a stream of tap water and then in distilled water, as pointed out by Richter (20). An agar so prepared will remain liquid down to about 34.5–35°C., and experience has shown that even the most delicate algal cells are uninjured by the short exposure to this temperature necessary in the plating process. From six to eight cc. of agar in a Petri dish eight cm. in diameter is a suitable quantity with which to plate. Larger quantities so thicken the layer of agar in the dish that the higher powers of the microscope, with their objectives of short focal length, cannot be used in locating small developing colonies.

Material to be Plated.—The alga to be plated should be collected with as little adhering foreign matter as possible. If it is a filamentous form which can be manipulated with a platinum needle, it can be materially cleansed by washing in sterilized nutrient solution such as is used in the preparation of the agar. If the alga is a unicellular form, little can be done in the way of preliminary cleansing. Dilutions are made in the usual manner, the degree depending upon the number of algal organisms present. The degree of dilution will depend in part, also, upon the number of bacteria and fungi present as determined by microscopic examination. It must be remembered that the algæ grow more slowly than most bacteria and fungi, and that unless the dilution, from the standpoint of the total number of organisms present, is great enough, the spread of bacterial and fungal colonies may be so great as to make the transfer of the later-appearing algal colonies impossible without contamination.

The material should be introduced into the tube of liquid agar while the latter is still a few degrees above its congealing point, in order that the inoculated tube may be vigorously shaken for some time before its contents are poured into the Petri dish. In this way the algal cells are freed of large numbers of either accidentally or regularly adhering bacteria.

Incubation and Transference.—The plates, after the agar has solidified, should be turned upside down in order to prevent the moisture which condenses on the cover from dropping, and spreading bacteria over the surface of the agar. Failure to do this often renders large numbers of platings worthless. The most favorable place to keep plates is in the light of a north window; and, as plates frequently remain under observation for many weeks, it is further desirable to have them in a glass case to prevent outside contamination. In general it is not advisable to cover the plates with bell jars, as it increases the humidity in the Petri dishes and accelerates the growth of moulds present as contaminations. The plates should be examined frequently and when rapidly spreading colonies of fungi or bacteria appear, these should be dissected out in order to save the remainder of the plate.

The length of time necessary for the appearance of the algal

colonies varies greatly with the species, from one to three or four weeks usually being required, depending upon the particular form. In most cases it is not possible to wait until the algal colonies can be seen macroscopically because spreading bacterial and fungal colonies usually encroach on the former to such an extent that a pure transfer is no longer possible. It becomes necessary, therefore, to look the plates over from time to time with the compound microscope in order to locate algal colonies in very early stages of development. For this purpose a 12 mm. objective is extremely serviceable, as its focal length is of sufficient magnitude to enable one to use it through the agar layer and glass bottom of a Petri dish and at the same time obtain a magnification considerably greater than that afforded by the ordinary low-power objective. The colonies located are conveniently marked by placing a small ink dot directly opposite them on the bottom of the Petri dish. Transfers should be made to agar slants by means of a minute platinum-foil spatula with which the agar directly over the ink dot can be neatly dissected out and transferred to the slant. It is not possible, in most cases, to make successful transfers with a platinum needle because the algal colony is usually composed of firmly cohering cells and, even in repeated attempts, not a single individual will adhere to the needle. Since many of the colonies are in the deeper strata, it is well to spread out the transferred agar fragment in a thin sheet in order to expose the contained algal cells directly to the air. Unless this is done, subsequent development in the slant may be extremely slow. Although bacteria grow slowly on this synthetic agar, their development is usually sufficient in a week to indicate whether the transfer has been successful or not. The purity of the culture may be further tested by making transfers to media more suitable for bacterial growth.

With this brief preliminary consideration of some of the more general phases of pure culture technique in the algæ, the isolation of single species will now be considered and attention called to the special problems and the technique involved in their isolation.

SPECIFIC

CHLOROPHYCEÆ

Chlamydomonas pisiformis Dill forma *minor* Spargo.—*Chlamydomonas* species frequently occur in water rich in organic materials, and teeming with bacteria. When the alga was in the resting condition, the mucilaginous cell walls were found so impregnated with bacteria as to render isolation in pure culture impossible. Platings with motile cells, however, showed that the latter were absolutely free from regularly adhering bacteria, but the number of bacteria present rendered the plates worthless. Then the gelatinous masses of resting cells were repeatedly washed with sterile water and finally placed in distilled water where, after twelve to twenty-four hours, zoöspores appeared in great abundance and congregated on the side of the vessel nearest to the light. A minute portion of this liquid containing the zoöspores was removed with a fine capillary tube and introduced into a tube of liquid agar and plated. In platings thus made, numerous colonies of *Chlamydomonas* appeared and the number of bacterial colonies was so small that a large number of successful pure transfers were made.

Where the number of available motile cells is small and it is important that isolations be made from these, a modification of the method used by Barber (3) in the isolation of yeasts and bacteria was frequently used to advantage. A large number of small, capillary pipettes were made and sterilized. After locating the cell or cells desired, they were removed with a pipette while being observed under the microscope, and transferred to a drop of sterile nutrient solution or water. This process was repeated until it was certain that the number of bacteria had been reduced sufficiently to admit of successful plating. They were then taken up again by means of a sterile pipette, transferred to a tube of liquid agar, and plated. Numerous pure cultures were obtained in this way.

Stichococcus bacillaris Näg., and *S. subtilis* (Kütz.) Klercker.—Preliminary platings with these forms showed that the cells, as obtained from the soil, yielded abundant bacteria-free colonies, and the problem of isolation became one of merely obtaining clean material and diluting sufficiently. Both of these species

of *Stichococcus* are soil-inhabiting and can be obtained—practically free from other algæ—on flower pots and greenhouse soils. The former species, because of its minute cells and the readiness with which the filaments resolve themselves completely into their constituent cells when placed in water, is a particularly easy one to obtain in pure culture. Rich material may be diluted until plates obtained from it show a sufficiently small number of bacterial colonies to admit of pure transfers and yet enough algal colonies for a number of transfers. *S. subtilis* is a larger species and the cells remain attached in rather long filaments. However, with vigorous shaking and previous teasing apart with needles, a sufficient number of single cells and small fragments of filaments are introduced to make possible numerous successful isolations. The washing of the cells to remove adhering bacteria can, in these species and many others, be largely accomplished by introducing the raw material into test-tubes containing sterile mineral nutrient solution or water, stoppering, and shaking vigorously. Direct transfers from these to liquid agar, or to tubes of sterile water for further dilution, may then be made. This procedure frequently enables one to make successful platings where the direct transfer of raw material to liquid agar results in constant failure.

Chlorella vulgaris Bey., and *Chlorella sp.*—Both of these species were isolated from soil in the open. An exterior gelatinous investment is, as in the two above mentioned species of *Stichococcus*, conspicuously absent, and preliminary experiments demonstrated that a large number of the vegetative cells were freed from all accidentally adhering bacteria by being shaken in the liquid agar before plating. The problem of isolating these species again becomes one of clean material and sufficient dilution. Species of *Chlorella* are perhaps the easiest among the algæ to isolate in pure culture, the process requiring little more than a direct application of bacteriological methods.

Attention should be called to another method—really a modification of the one just given—by means of which *Chlorella* species may be obtained in pure culture. Its application is not necessary in the species of *Chlorella* investigated, since

the vegetative cells can be so readily freed from adhering bacteria. But its general applicability to other forms justifies its mention at this place. *Chlorella*, like many other genera of the *Protococcales*, forms non-motile endogenous daughter cells which remain enclosed in the mother wall for varying lengths of time. The enclosed daughter cells are in all cases free from adhering bacteria. A group of daughter cells still enclosed within the mother membrane may be removed by means of a capillary pipette to a drop of sterile water, and from here to a succession of others until all readily removable bacteria have been left behind. The last transfer should be made to a drop of sterile water on a small sterile cover glass. By a slight pressure of a second cover glass, the mother membrane may be ruptured, liberating the enclosed, bacteria-free cells. The two cover glasses should then be introduced into a tube of liquid agar, the latter shaken vigorously, and finally poured into a Petri dish. Frequent isolations have been made in this way, and its importance in forms whose vegetative cells cannot be freed from adhering bacteria, and which do not form motile spores but only non-motile endogenous daughter cells, can hardly be overestimated.

Pleurococcus vulgaris Menegh.—The majority of *Pleurococcus* cells, when thoroughly washed, will be found free from bacteria. A difficulty which frequently arises is that the alga grows so very slowly that fungi—which are persistently present in *Pleurococcus* cultures—take entire possession of the plates before a transfer can be effected. But with careful searching, minute colonies—often consisting of but a few cells—can usually be found and successfully transferred. The transferred colony, however, usually makes extremely slow progress in its growth on agar. Much better results are obtained when transfers are made to evaporimeters (as devised by Livingston (15)) supplied with the mineral nutrient solution.

Scenedesmus sp., and *Kirchneriella sp.*—Both of these species were obtained in pure culture by washing and diluting clean, concentrated material in sterile mineral nutrient solution, and then plating. The great majority of the colonies of both species were contaminated with bacteria, pure colonies being very rarely found. This fact, together with the gelatin-

ous exterior characteristic of the cells of both species, makes it probable that the pure colonies developed, not from mature individuals, but from autocolonies (produced within mature cells) which either had just escaped from the mother cell or had done so during the vigorous shaking,—in either of which cases they are free from adhering bacteria.

Chlorococcum humicola (Näg.) Rabenh.—This species was isolated in the zoösporic condition. The alga, collected from soil, was placed in sterile mineral nutrient solution and after twenty-four hours produced zoöspores in abundance. Platings with these yielded numerous pure colonies from which successful transfers were made. In this connection it should be mentioned that all zoöspores thus far experimented with—including a considerable variety of forms—have been found free from bacteria. It is needless to say, therefore, that the presence of zoöspores in the life cycle of any alga provides a logical point of attack for its isolation in pure culture. While not all the attempts to isolate zoösporic forms in pure culture have proved successful, it is entirely probable that they will when the general technique is more closely adapted to individual forms.

Protosiphon botryoides (Kütz.) Klebs.—The vegetative plant of *Protosiphon*, with its root-like process extending into the soil and the large aerial portion, is so persistently covered with bacteria that its isolation in pure culture in this condition is quite impossible. With slight desiccation, however, large numbers of chlamydospores with dry non-gelatinous membranes appear, which, at least so long as they remain enclosed within the mother membrane, are free from bacteria. From these, isolations in pure culture can be readily made according to the second method suggested for *Chlorella*—by carefully washing an individual plant filled with chlamydospores, liberating the latter by teasing with needles or by a slight pressure of the cover glass, and plating in the usual manner. Another method which has yielded pure cultures, but which is not to be recommended because it is far less reliable than the one just described, is based on the use of the motile gametes. When vigorous *Protosiphon* plants, growing on soil, are covered with distilled water, gametes, which congregate in the lighted

side of the vessel, are produced in large numbers. Plates made with this material yield an occasional pure culture, but most of the gametes fail to develop. It is impossible at present to say whether the colonies develop from newly formed zygotes or from gametes which fail to conjugate.

Stigeoclonium tenue (Ag.) Kützing.—The ease and certainty with which zoöspores can be induced to develop in this form, and their extreme abundance, makes it, although a filamentous alga, an especially easy one to isolate. Freshly collected and thoroughly washed filaments of *Stigeoclonium*, placed in distilled water or sterile nutrient solution, will, in from twelve to twenty-four hours, develop a great abundance of zoöspores. Cultures prepared in this way contain so small a number of bacteria that plates containing a hundred or more *Stigeoclonium* zoöspores are sufficiently free from bacterial colonies to render numerous successful pure transfers possible. Although a filamentous form, *Stigeoclonium* grows exceedingly well on the mineral nutrient agar. While other members of the *Chætophoraceæ* were not experimented with, it is reasonably certain that forms like *Microthamnion*, *Chætophora*, and *Draparnaldia*, all of which readily yield large quantities of zoöspores, may be obtained in pure culture by a method identical with or similar to the one employed in the isolation of *Stigeoclonium*.

Oedogonium sp., and *Vaucheria sp.*—While neither of these forms were obtained in pure culture, the observations made render it altogether likely that this will be possible when a little more attention is given to the cultural solutions. Repeated trials with the vegetative filaments demonstrated that from the latter no pure cultures could be obtained directly. The oöspore proved equally unsatisfactory because the oögonial wall is covered with adhering bacteria. Again, the oöspore is, in most cases, so firmly and completely united with the oögonial wall that its separation from the latter is at present impossible. In both forms, however, zoöspores are readily obtained, and preliminary experiments demonstrated that these, like zoöspores in general, are bacteria-free. Where zoöspores could not be obtained in large quantities, individual ones were isolated with sterile pipettes, washed repeatedly in sterile water, and then either plated in the usual manner, or introduced into a

tube of sterile mineral nutrient solution. Although the great majority of such isolations remained bacteria-free, the zoöspores failed to develop, and finally died. It is only necessary, therefore, to find some medium in or on which the zoöspores will germinate and develop into plants, to effect a pure culture of *Vaucheria* or *Oedogonium*. *Bulbochæte* was not used, but in all probability this form will lend itself to a similar technique.

Conjugales.—Thus far it has not been possible to obtain a pure culture of any member of the *Conjugales*. The representatives of this order, in their vegetative phases, are provided throughout with an exterior gelatinous investment which is very generally impregnated with bacteria. All attempts to obtain pure cultures from vegetative individuals failed. Further, there is a complete absence in the order of motile spores and, in general also, of separable, asexual, endogenous spores. The zygospore, therefore, suggests itself as a possible means of solving the problem, especially in those forms where it is produced endogenously, and where it does not subsequently coalesce with the wall of the gametangium. While pure cultures were not obtained from these, the method used in *Spirogyra setiformis* is of interest and may prove serviceable in the ultimate isolation of these forms in pure culture.

Filaments containing mature zygospores, but in which the zygospore-containing cell walls were still completely intact, were washed repeatedly in sterile water and then broken up as thoroughly as possible with needles; in this process, numerous zygospores were freed from the enclosing walls, later to be taken up with sterile pipettes, and transferred to sterile drops of water. Each zygospore was subsequently transferred from ten to twenty times to fresh, sterile water drops, and finally taken up with a sterile pipette. When a considerable number of zygospores had thus been isolated, they were introduced into a tube containing a few cc. of sterile water, vigorously shaken, and the entire contents poured out into a Petri dish containing a layer of sterile nutrient agar. After rocking the dish for a short time, it was allowed to remain quiet until the zygospores had settled down on the surface of the agar. The free water was then very slowly and carefully, but completely, drained from the surface of the agar, and the plate allowed to remain

in the light. While in a few cases bacterial colonies developed about the zygopsores, it was found that the great majority were free from all adhering bacteria. Such zygospores as were bacteria free were then transferred to test tubes containing sterilized mud and pond water. Although about sixty such transfers were made, not a single one yielded a growing culture, although zygospores kept in battery jars in the laboratory showed a high percentage of germination. It will require further experiments to find a suitable medium for the germination and subsequent growth of isolated zygospores. However, the isolation of bacteria-free zygospores justifies the opinion that with them it will, sooner or later, be possible to culture *Spirogyra* in a state of purity.

HETEROKONTÆ

Botrydium granulatum (L.) Greville.—This form is, in its general morphology, so similar to *Protosiphon*, that the technique, as regards the use of chlamydospores, described for the latter, is entirely applicable here. *Botrydium* when submerged, however, forms an abundance of zoöspores instead of gametes, and from these pure cultures can be obtained with great ease when plated in the usual manner. The method for using the chlamydospores can also be considerably abbreviated in *Botrydium*. When the plants form chlamydospores, the aerial globular portion of the plant collapses. The cell, however, is so large that the aerial bag can be torn open with fine sterile forceps, the spores removed under a hand lens with a needle and transferred directly to liquid agar. Platings made in this way show a very slight bacterial contamination, and pure transfers can be made in abundance. While a direct, bacteria-free transfer has not been thus effected, it is altogether probable that it can be done. The pure transfers of *Botrydium* having been obtained, it was found that their development on agar was extremely slow, and ultimately all of the cultures died. Further experiments will be necessary in order to provide a favorable medium for growth. The clay-cup evaporimeter may perhaps prove of service in this connection as it did in the case of *Pleurococcus*.

Botrydiopsis sp.—This form was found abundantly during

one season on soil in the greenhouses. The vegetative cells when placed in water readily produce zoöspores, and isolations were made from these with little difficulty. Unlike *Botrydium*, this form grows exceedingly well on the mineral nutrient agar.

BACILLARIALES

The diatoms were encountered only incidentally in connection with other forms, and no particular effort was made to isolate forms in pure culture. Although diatoms, in general, have a gelatinous exterior, a small *Navicula* was on several occasions obtained in pure culture and grown successfully. It should be said, however, that the great majority of diatom colonies obtained were contaminated with bacteria.

CYANOPHYCEÆ

In the class *Cyanophyceæ*, the most difficult problems of isolation are met. The almost universal presence of an abundance of external mucilaginous material, the complete absence of ciliated reproductive cells, and the virtually complete absence of free, endogenous spores, renders the technique particularly difficult. The gelatinous investments are, in all cases investigated, impregnated with bacteria which cannot be completely removed by the most vigorous washing. Among the forms studied were *Aphanocapsa*, several species each of *Oscillatoria*, *Nostoc*, and *Anabæna*, *Cylindrospermum*, and *Microcoleus*. Of these, only two species, one of *Oscillatoria* and one of *Microcoleus*, were obtained in pure culture.

In the isolation of these two forms, silicic acid jelly was found to be indispensable. While directions for preparing this medium are to be found in many places in the literature, certain difficulties encountered in its preparation have made it desirable to give at this time, and in some detail, the method used.

As regards the preparation and mixing of the sodium silicate and hydrochloric acid solutions, the directions given by Smith (22) may be followed. It is only necessary to point out in this connection that if Merck's "sodium silicate pure crystals" is used, the solution should be made up with cold water. If hot water is used, an unidentified substance (insoluble in cold water) goes into solution, and frequently causes the coagulation

of the silicic acid-hydrochloric acid mixture before dialysis is complete. A point of very great importance is the preparation of the collodion dialyzing bags. As has been pointed out by Kellerman (12), and others, the degree of permeability of the bags depends, in a large degree, upon the way in which they are made. If the guncotton solvent is made from equal parts of ether and absolute alcohol, the bags will, in most cases, have a very low permeability, and coagulation of the enclosed silicic acid solution will frequently result before dialysis is complete. The degree of impermeability is further increased by drying the bags rapidly. If, however, 95 per cent (instead of absolute) alcohol is used, and the bags are allowed to dry spontaneously by inverting the test-tubes in which the bags are being prepared in suspended wire baskets, a much higher degree of permeability will be obtained.

Bags prepared with 95 per cent alcohol were used, and the silicic acid-hydrochloric acid mixture dialyzed in tap water until the chloride content was no greater than that of the water. The silicic acid solution was further purified by dialyzing in changes of ordinary distilled water and finally in triply distilled, nitrogen-free water. In this extended dialysis, a considerable portion of the silicic acid is lost, and it usually becomes necessary to concentrate the solution to obtain a jelly of sufficient firmness. This is best carried out in heavy, two-liter suction-flasks in which the pressure is reduced until the solution boils at from 35 to 40°C. If the concentration is carried out at higher temperatures, coagulation sometimes results. In order to prevent the violent bumping which always takes place unless some special precautions are taken, it is only necessary to bring through the rubber stopper at the top of the suction-flask a glass tube drawn out at the bottom to a very fine capillary, which dips into the solution. The top of this tube, outside of the rubber stopper, should be provided with a piece of rubber tubing and pinch cock to regulate the intake of air. The air thus admitted may first be washed to remove carbon dioxide, ammonia, or other impurities. The concentration should be continued until a sample, when congealed, has the proper consistency. The directions given by Smith (22) for coagulating the medium apply here and it need only be mentioned

that the concentration of the mineral nutrients employed in the agar, 0.1 per cent, is quite sufficient to bring about coagulation.

After it had become probable that no blue-green alga, in the ordinary vegetative condition, could be isolated by the usual plating method, tubes containing from two to three inches of solid, sterile, synthetic agar were inoculated at the surface with a species of *Oscillatoria*. The tubes were then completely wrapped in black paper, leaving only the very bottom exposed to the light, and inverted. It was hoped that in the rapid growth of the alga through the agar, the bacteria might be left behind. The growth toward the light in some cases amounted to eight mm., and more, per day. When the growth had approximately reached the bottom of the tube, the end of the latter was broken away, the surface of the agar seared, and transfers made from the interior of the agar plug. Although the experiment was repeated many times, and a total of at least fifty transfers made, a pure culture was never obtained, bacteria always being present. Large Petri dishes, containing a layer of sterile synthetic agar, were then inoculated at one edge with a species of *Oscillatoria*, and the dishes so placed that the point of inoculation was farthest away from the light. The alga grew rapidly (on the surface of the agar) toward the light, and just before reaching the opposite edge of the dish, transfers were made from the farthest advanced filaments. Although transfers to fresh agar surfaces were continued to the number of six, a pure culture was never obtained.

The experiment was then repeated, surfaces of silicic acid jelly replacing those of agar, with the result that numerous pure transfers were obtained from the second plate. A species of *Microcoleus* was obtained in pure culture in an identical manner.

Most members of the *Oscillatoriaceæ* are provided with a sharply delimited, gelatinous sheath. Reproduction is effected by the formation of hormogonia which glide out of the sheath, move about slowly for a time, and then come to rest. In forms like *Microcoleus*, *Lyngbya*, and some species of *Oscillatoria* in which the hormogonia escape from definite sheaths,

leaving the latter behind, it is fairly certain that the hormogonium is originally free from bacteria, but becomes contaminated in passing through the older portion of the empty sheath and out of its terminal opening, both of which are more or less infected with bacteria. The persistence with which the bacteria cling to the hormogonium of *Oscillatoria*, once having infected it, is clearly shown by cultures on agar surfaces. Although a single hormogonium may have moved as much as two inches away from its parent filament, creeping all the while over a sterile agar surface, the hormogonium will be found covered with bacteria, and the path over which it moved will be clearly indicated by a continuous, linear colony of bacteria. With the use of silicic acid jelly, however, the multiplication of the bacteria is reduced to such an extent that, after a time, hormogonia escape uncontaminated, and begin the development of pure colonies. Transfers from these, however, grow very slowly and in most cases eventually die. It seems probable, when *Oscillatoria* and *Microcoleus* have been completely separated from the invariably present bacteria, that the media which were favorable in the presence of the bacteria, become unfavorable in their absence. Further work will be necessary to grow these forms successfully after they have been isolated in pure form. The silicic acid jelly method was also attempted with the above mentioned heterocystic forms; however, up to the present time, no successful isolations have been made.

DISCUSSION

It is apparent that the technique involved in the isolations just referred to depends entirely on mechanical separation of one kind or another. This method is reasonably efficient in those species in which zoöspores or other free endogenous spores are readily obtainable, or in which vegetative cells are either free from bacteria or can be rendered so by mechanical means. It is true that even in some species forming free endogenous spores, the above methods have not yielded pure cultures, as, for instance, in *Vaucheria*, *Oedogonium*, and *Spirogyra*. In these cases, however, it should be pointed out that it is not the isolation technique which is at fault but rather

the cultural methods. Zoöspores and zygospores, respectively, free from other organisms, were obtained in these cases but failed to develop in the cultural media subsequently supplied. There can be little doubt, however, that the latter difficulty will be overcome in time.

Except in the *Oscillatoriaceæ*, little progress was made in the *Cyanophyceæ*. The problem appears especially difficult in the *Coccogoneales* where all forms of motile reproductive bodies are absent, and in which the vegetative cells apparently cannot be rendered free from adhering organisms by mechanlica means. Even in the heterocystic *Hormogoneales*, the situation is a difficult one, the more slowly moving hormogonia apparently being unable to escape the bacteria.[1] While no experiments were made along these lines, it appears highly desirable to attack the problem in the latter group through the spore. It is well known that the spores of blue-green algæ are extremely resistant to heat, and it does not appear improbable that the bacteria—especially if they are all in the vegetative condition—could be killed by heat, leaving the algal spores unharmed. Chemical sterilizing agents may also prove of value here. The latter may also prove serviceable with members of the *Coccogoneales* and certain of the grass-green algæ which have thus far failed to yield to the technique employed.

Conclusions

1. By adapting methods of pure culture technique to individual species of algæ, it has been possible to isolate in pure culture the following forms:

Chlorophyceæ.—Chlamydomonas pisiformis Dill forma *minor* Spargo, *Stichococcus bacillaris* Näg., *S. subtilis* (Kütz.) Klercker, *Ulothrix sp.*, *Chlorella vulgaris* Bey., *Chlorella sp.*, *Pleurococcus vulgaris*, *Scenedesmus sp.*, *Kirchneriella sp.*, *Chlorococcum humicola* (Näg.) Rabenh., *Protosiphon botryoides* (Kütz.) Klebs, *Stigeoclonium tenue* (Ag.) Kützing, and a number of others of uncertain identity.

[1] In a contribution which has just appeared (Kulturversuche mit Chlorophyll-führenden Mikroorganismen, III. Zur Physiologie der Schizophyceen. Beitr. z. Biol. d. Pflanzen 12: 49–108. 1913), Ernest G. Pringsheim reports the isolation in pure culture of a species of *Nostoc*. The method used was that of repeated transfers to sterile silicic acid jelly plates.

Heterokontæ.—Botrydium granulatum (L.) Greville, and *Botrydiopsis sp.*

Bacillariales.—Navicula sp.

Cyanophyceæ—Oscillatoria sp., and *Microcoleus sp.*

2. In addition, zoöspores from *Vaucheria* and *Oedogonium*, and zygospores from *Spirogyra* have been isolated free from other organisms.

In conclusion, the author wishes to express his gratitude to Dr. Geo. T. Moore, at whose suggestion the work reported herein was undertaken, whose advice and interest have been a source of constant help; and to Mildred Spargo Schramm, for kindly assistance in many ways.

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THE IDENTIFICATION OF THE MOST CHARACTERISTIC SALIVARY ORGANISM, AND ITS RELATION TO THE POLLUTION OF AIR[1]

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Introduction

Bacteriologists and sanitary engineers have, within the last score of years, given much attention to the detection of excremental pollution in water. They have shown that by making it possible to recognize certain characteristic accompanying organisms, bacteriological methods are capable of revealing this kind of pollution even when it exists to such a small degree as to be beyond the range of chemical detection. Small as these quantities of contaminating substances may seem, they may nevertheless endanger the health of a whole community by exposing it to possible pathogenic organisms derived from the excreta of a diseased host.

It is not merely by the aggregate bacterial yield that the potability of a water in its relationship to disease is judged, but more specifically by the species of bacteria present, and their relative abundance. The micro-organisms which serve as an index of pollution, and for which special quantitative examination is made, are the members of the colon group. These, from their constant presence and relative abundance, are characteristic of material of excremental origin. Their presence in water in sufficient quantity indicates pollution, and their relative abundance serves as an index to the extent of the latter.

Bacteriological technique has not as yet been applied to the same extent in the detection of pollution in air. Chemistry has, up to the present time, been of more practical value here.

[1] An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment of the requirements for the degree of master of arts in the Henry Shaw School of Botany of Washington University.

The proportion of carbon dioxide is still the standard mainly relied on for estimating pollution of air by materials given off from the human body, although it is recognized that other factors may be of more importance. This method of examining air, however, is of little or no value in furnishing an index to the probable or possible contamination with disease-producing germs, for there is at present no reason for believing that such organisms are given off in the breath during ordinary quiet breathing. Thus, M. H. Gordon[1] calls attention to the following: Tyndall observed that expired air is optically purer than inspired air; Cornet found air expired by tubercular patients to be free from the tubercle bacillus; and Straus has shown that expired air is not only comparatively free from bacteria, but that it is considerably purer in this respect that inspired air. It nevertheless appears probable that bacteriology rather than chemistry will furnish a means of investigating the pollution of air by disease-producing germs. The problem at hand is to devise, if possible, a method for estimating the degree of pollution of air by pathogenic organisms (given off from the human body) in a manner similar to that employed in estimating the extent of pollution of water by similar organisms of excremental origin.

HISTORICAL

It appears that the present status of bacteriological analysis of air is comparable to that of bacteriological analysis of water some years ago, when the total number of bacteria in a given quantity was the chief factor determined. There are various ways in which pathogenic organisms may gain access to the air and ultimately to another individual. In addition to transfer by direct contact, disease-producing organisms may be given off in the urine, in feces, in sputum, or from the surface of the skin. Recently, also, attention has been called to the possibility of the pollution of air by the scattering of fine particles of mucus and saliva from the mouth in the acts of coughing, sneezing, and loud speaking. The latter methods of air pollu-

[1] Report on a bacterial test for estimating pollution of air. Supplement to the Thirty-second Annual Report of the Local Government Board (London), containing the Report of the Medical Officer for 1902–3. 421–471. 1904.

tion are the ones to be considered in this investigation. They doubtlessly constitute an important means whereby pathogenic organisms enter the air from an infected person, subsequently to be transmitted to other individuals.

The discharge of sputum furnishes the most obvious way whereby pathogenic organisms may be expelled from the mouth. The expectorated mucus dries, and, in the form of dust, may later be inhaled to produce infection. The work of Flügge and members of his school,[1] however, has drawn attention to a more direct and no less important way by which germs may be aërially conveyed from the mouth. The problem of transmission of micro-organisms by means of particles of mucus expelled from the mouth in various expiratory acts, was attacked in two ways by the investigators referred to above: 1. The mouth was artificially infected with a culture of *Bacillus prodigiosus*. This organism was chosen because the red pigmentation of the colonies renders the identification easy. After agar plates had been placed at various distances from the person being experimented upon, the individual proceeded to speak, cough, sneeze, etc. At the end of the experiment the agar plates were covered and incubated at 25° C. for 3 days, during which time the characteristic red colonies of *B. prodigiosus* made their appearance. The possibility of error due to the previous presence of this organism in the air of the room was excluded by exposing a series of agar plates immediately before the experiment began, with the result that in all cases the organism failed to appear. The length of time that droplets of mucus remained suspended in the air after the several expiratory acts was determined by exposing plates at various periods after the experiment had been completed. 2. Glass slides or empty Petri dishes were placed at various distances from a tubercular patient. The droplets of mucus expelled during coughing, and deposited upon the glass slides, etc., were either examined microscopically or were washed off and injected intraperitoneally into guinea-pigs. In the former case a bacillus giving the characteristic staining reaction of the tubercle bacillus was found, and in the latter the development of tuber-

[1] Gordon, *loc. cit.*

culosis in the inoculated animals resulted. In other experiments, guinea-pigs, instead of being inoculated, were directly exposed to the coughing of tubercular patients with the result that a number of the animals so exposed contracted tuberculosis. Varied and repeated experiments along these lines established the fact that in the acts of coughing, sneezing, and loud speaking, fine droplets of mucus are ejected into the air, that they float about and may be wafted by air currents, such as obtain in ordinary rooms, to a distance of from 24 to 40 feet.

The most thorough investigation in recent years of the problem of air pollution with micro-organisms was made by Dr. M. H. Gordon.[1] This author believed that the positive recognition of disseminated saliva constituted an important step in the development of an applicable bacteriological method for the examination of air. By bacterial analyses of a number of samples of saliva obtained from normal individuals, Dr. Gordon determined that the streptococci are the organisms most abundantly present in saliva. Of these he was able to differentiate four morphologically different types—*longus, mediius, brevis,* and *conglomeratus*. In endeavoring to differentiate these organisms on a physiological basis a study was made of their virulence, relation to oxygen, optimum growth temperature, pigment production, motility, gelatin liquefying power, indol production, action on litmus milk at 37°C., and action on various carbohydrates.

It was found that the micro-organism which is most useful in the detection of droplets of saliva is *Streptococcus brevis* because it is the only one among the salivary cocci found which changes the color of neutral red broth to yellowish green, and produces acid and clot in milk. Having developed a means of differentiating the coccus most characteristic of saliva, Gordon next examined the open air for the presence of micro-organisms characteristic of saliva. In these experiments broth plates were exposed for a stated length of time and incubated anaërobically at 37°C. In but very few cases were the organisms isolated from the air.

A further means of differentiating the characteristic salivary

[1] *Loc. cit.*

coccus from the air cocci was sought in the action of the two on various organic substances. In this capacity the several broths containing lactose, syringin, and coniferin, proved especially serviceable. In lactose broth the typical salivary coccus was positive, i. e., it produced acid, whereas the air cocci were negative. In the syringin and coniferin broths, the air cocci were positive, the typical salivary coccus negative.

To determine whether or not particles of saliva were disseminated through the air during the acts of coughing, sneezing, and loud speaking, Gordon performed experiments in a large and in a small room, using, at first, Flügge's method of artificially infecting the mouth with a living culture of *Bacillus prodigiosus* and placing sterile agar plates at various distances in front of and behind the speaker. After $\frac{1}{2}$–1 hour of loud speaking, it was found that *B. prodigiosus* had been disseminated to a distance of 40 feet in front of and of 12 feet behind the speaker. In other experiments in which no artificial infection of the mouth was resorted to, but in which the characteristic salivary coccus served as the index of dissemination, it was found that after $\frac{1}{4}$–1 hour of loud speaking *Streptococcus brevis* appeared on broth plates placed as many as 12 feet in front of and behind the speaker. In similar experiments in which speaking was continued for one hour in an ordinary conversational tone, no dissemination of the salivary *Streptococcus* could be detected.

From his experiments Dr. Gordon inferred that there were certain streptococci normally present in saliva which are applicable for the detection of droplets of saliva in air in much the same manner that *Bacillus coli* can be applied for the detection of fecal matter in water.

The Identification of the Most Characteristic Salivary Organism

With a view of determining the organism most characteristic of saliva, I have undertaken, as a first step, a bacteriological analysis of the saliva of a normal individual. In this examination special attention was paid to the type of organism most abundantly present. Having determined the type, i.e., whether bacillus, coccus, or spirillum, characteristic reactions for it were next sought in order to render its recognition easy. Since

a possible relation of the characteristic salivary organism to the pollution of air was to be investigated, it was necessary to examine the outdoor air free from human contamination for the presence of micro-organisms closely allied to those characteristic of saliva. As particles shed from the skin may be present in the air, it was further necessary to examine those micro-organisms found on the skin which were closely allied to the ones characteristic of saliva.

In examining the saliva for the type of micro-organism most constantly present, i.e., whether bacillus, coccus, or spirillum, the dilution method was used. It is reasonably safe to assume, after repeated trials, that the type of micro-organism which persists longest in continued dilutions is the type most abundant in the material examined. This is true provided the medium on which the organism is grown is approximately equally favorable for the development of all the types present. The dilutions were carried out as follows: A sample of saliva was collected in a sterile test-tube and 1 cc. introduced into a second tube containing 9 cc. of sterile distilled water. The contents of the latter were then thoroughly mixed and 1 cc. of the liquid introduced into a third tube likewise containing 9 cc. of sterile distilled water. This procedure was repeated until 6 dilutions had been effected. Obviously, 1 cc. quantities of each of the 6 successive dilutions contain respectively $\frac{1}{10}$, $\frac{1}{100}$, $\frac{1}{1,000}$, $\frac{1}{10,000}$, $\frac{1}{100,000}$, and $\frac{1}{1,000,000}$ cc. of saliva.

One plate each from dilutions 4, 5, and 6 was made, 1 cc. of the respective dilutions being introduced into 10cc. of nutrient + 1 agar. After thorough mixing, the plates were incubated aërobically for 24 hours at 37°C. The plate made from dilution 5 produced 20 colonies, whereas the one from dilution 6 showed no growth. From each of the 20 colonies a cover-glass preparation stained with gentian violet was made. Microscopic examination revealed the fact that each of the 20 colonies was composed of micro-organisms of the coccus type. Transfers were then made to agar slopes which were incubated at 37°C., for 24 hours. The cultures obtained in this manner were numbered from 1 to 20 and kept at 20°C., as stock cultures.

In examining the open air, sterile agar plates were exposed as indicated in table 1.

TABLE I

DATA ON THE COLLECTION OF AIR COCCI

Place of exposure.	Time of exposure	Total colonies on plate after 24 hrs. at 37°C.	Coccus colonies on plate after 24 hrs. at 37°C.	Number given to stock culture	Remarks
Window sill outside of laboratory, 2nd floor.	15 minutes.	14	7	21 to 27 incl.	
On shelf, center of laboratory room.	do.	2	0		One person in room. Abundance of *Monilia* present.
On table, in reading room.	do.	2	0		do.
On table, in plating room of laboratory.	do.	0	0		*Monilia* suppressed growth.
On table, in basement	do.	3	0		
On lawn, in garden	do.	9	4	28 to 31 incl.	
In living room.	do.	7	6	32 to 37 incl.	
Window sill, 4th floor, downtown section	do.	18	0		Much soot on plate.
On table, in draughting room.	do.	1	1	38	One person in room.

After exposure the plates were covered and incubated aërobically for 24 hours at 37°C. Stained preparations of all colonies developing were made and examined under the microscope. The coccus forms were transferred to agar slopes, and after incubation for 24 hours at 37°C., were kept in stock at 20°C. Several of the plates exposed in various parts of the laboratory building were rendered worthless by an abundant growth of *Monilia sitophila*.

The method of examining the skin for organisms closely allied to those characteristic of saliva, was as follows: Test-

tubes, each containing 10 cc. of distilled water and a piece of linen 2 inches square, were sterilized in the autoclav for 15 minutes at 15 pounds pressure. Samples were taken from three parts of the body of a normal individual, namely, the calf of the leg, the thigh, and the chest. This was accomplished by briskly rubbing the portion of the body from which the sample was to be taken with the piece of linen held in sterilized forceps, and later replacing it in the tube of sterilized water. From these dilutions, after being thoroughly shaken, about ½ cc. quantities were plated in 10 cc. of nutrient agar. From each plate 2 coccus colonies were selected from which transfers were made to agar slopes. These, after 24 hours at 37°C., were kept as stock cultures at 20°C.

There were now in stock a total of 44 pure cultures of *Coccaceæ*, 20 from saliva, 18 from the open air, and 6 from the skin.

MORPHOLOGICAL CHARACTERS

The form of the individual cell is of little value in differentiating the species of *Coccaceæ*, for under conditions favorable to their growth, all appear as regular spheres. Irregular oval cells occur at times, but the form usually becomes normal after cultivation. Some writers lay considerable stress on the value of cell grouping in the *Coccaceæ* as a means of differentiation. With the utmost care in cultivation and staining, however, this could not be verified in the cultures under observation. All the cultures examined contained cells occurring singly, in pairs, in short chains, and in masses, but in no case did the cells of any specific culture exhibit a distinct tendency to occur in any one form. A stained cover-glass preparation showed various cell groupings in different parts of the same microscopic field.

Cell grouping was studied in the following manner: An oese of sterile +1 bouillon was placed on a sterile cover glass, inoculated with a 24-hour culture of the organism to be examined, and inverted on a Van Tieghem cell containing a few drops of sterile distilled water. After sealing the cover glass on the cell with vaseline, the preparation was incubated for 24 hours at 37°C. At the end of this time the cover glass was removed and the drop of water containing the organism allowed to

evaporate. Then, 3 drops of mercuric chloride solution were applied and after 2 minutes washed off with distilled water. Following this, the preparation was treated with a few drops of 1 per cent acetic acid for 5 minutes, again washed in water, and finally stained for about 15 seconds with a few drops of gentian violet. After washing, and drying in the incubator at 37° C., the vaseline was removed from the cover glass with xylene, and the preparation mounted in balsam and examined under the microscope. The relation to Gram stain was observed on 2 and 4-day agar cultures incubated at 20°C. The preparations were treated with aniline oil-gentian violet for $1\frac{1}{2}$ minutes, with Gram's iodine solution for $1\frac{1}{2}$ minutes, and finally with 95 per cent alcohol for 3 minutes. The reactions are recorded as "− −" (decolorized in both tests), "+ −" (stained in one test and decolorized in the other), and "+ +" (stained in both tests).

CULTURAL CHARACTERISTICS

All cultural characteristics were observed in streak cultures on agar slants after 14 days' incubation at 20°C., and 37°C. Such differences as developed between the cultures were almost entirely variations in color and vigor of surface growth. Under the latter, 5 types were distinguished as follows:

1. Growth very faint and veil-like, or forming scattered translucent colonies.
2. Growth better, but still meager.
3. Growth good, but not abundant.
4. Growth abundant.
5. Growth very heavy.

In the study of chromogenesis, apparent differences in pigment production, due to unequal vigor of growth or evaporation, were, so far as possible, eliminated. This was accomplished by examining in each case the same amount of material—a loopfull—spread evenly on white drawing paper having a rough surface. After drying at room temperature, the color of the pigment produced was compared with the colors as given by Ridgway[1].

[1] Color standards and nomenclature. 1912. [Published by the author, Washington, D. C.]

This author uses as a basis the solar spectrum with its six fundamental colors and intermediate hues, augmented by a series between violet and red not in the spectrum.

BIOCHEMICAL REACTIONS

The production of indol was investigated in 5-day peptone broth cultures incubated at 37°C. One cc. of a 10 per cent sulphuric acid solution was thoroughly mixed with the broth culture, and then 1 cc. of a freshly prepared 0.01 per cent solution of sodium nitrite was carefully run in on top of the mixture. The appearance of a pink ring at the juncture of the nitrite solution with the acid-peptone solution, was regarded as an indication of the presence of indol. A blank determination for purposes of comparison was made in each case. The action on neutral red broth as regards change in color was observed in cultures incubated for 5 days at 37°C., in the presence of hydrogen.

The organisms were further grown in solutions of nitrate broth to determine whether or not reduction takes place, and if so, whether to nitrite or to ammonia. In carrying out the test a tube of nitrate broth was inoculated with the organism to be tested, and incubated for 4 days at 37°C., an uninoculated tube of nitrate broth being similarly treated to serve as a check. At the end of 4 days, 3 cc. of the broth were removed to a clean test-tube, and 2 cc. each of a naphthylamine solution and of a sulphanilic acid solution added. The development of a red color indicates the presence of nitrites, the intensity of the color being proportional to the amount of nitrites present in solution. To test for ammonia in the remaining portion of the culture, a few drops of Nessler's solution were added. The appearance of a yellow color or precipitate indicates the presence of ammonia. In studying the liquefaction of gelatin by the cocci under observation, the extent of the action only was determined. This was accomplished by spreading a suspension of the organism over the surface of gelatin in 10 mm. tubes. It was found that the amount of material used in this inoculation did not affect the total amount of liquefaction, i.e., whether the amount of transferred material was large or

small the extent of the liquefaction after 30 days' growth at 20°C., was the same with any one organism.

In the study of the action on sterile certified milk particular attention was paid to the coagulation of the milk and to the production of acid. Observations were further made on the effect of the organisms on lactose, saccharose, mannite, salicin, inulin, sorbite, raffinose, and rhamnose. The medium in which these organic substances were used was prepared according to Dr. Houston's formula, as follows:

Liebig's beef extract	1.0 per cent
Peptone	1.0 per cent
Organic compound to be tested	1.0 per cent
Sodium bicarbonate	0.1 per cent
10 per cent litmus solution	1.0 per cent

The medium, neutral in reaction to litmus, was sterilized for 15 minutes at 15 pounds pressure in 500 cc. containers, from which sterile fermentation tubes, provided with glass caps, were directly filled. In doing this it was necessary to take utmost precautions to obviate any possibility of contamination. The various organic media thus prepared were inoculated, not from an agar slope, but from a 48-hour broth culture. Gas formation and the production of acid in the several media were observed after 3 days' incubation at 37°C.

DISCUSSION OF RESULTS

A thorough study of the results will now be made with a view of finding, if possible, some characteristic or group of characteristics, morphological or biochemical, which may be used in differentiating the salivary cocci from the coccus forms of the air and the skin.

The cell grouping varies throughout, there being no arrangement characteristic of any particular group. As observed, all the forms occur in groups, chains, and pairs. As regards the deportment of the various organisms toward the Gram stain, it was noted that all of the salivary cocci gave a positive reaction in both tests; of those from the air, 3 were positive in the two tests, 8 alternately negative and positive, and 6 negative throughout; of the skin cocci, 4 were positive and 2 negative

in both tests. This stain, as may readily be seen, is of no differential value here, for, although the salivary cocci react positively throughout, both positive and negative reactions occur among the air and skin forms.

The production of indol among coccus forms is very uncommon. Of the salivary cocci under observation, none produced indol, and of the air and skin forms only one from each group produced it. The change of color in neutral red broth is, apparently, more frequently brought about by the salivary cocci than by the air and skin forms, but this difference is not sufficiently well marked to be of differential value. Of the 20 salivary cocci, 12 produced fluorescence, whereas only 1 of the air and none of the dermal forms produced this change. All of the forms under observation reduced nitrates to ammonia. Of the salivary forms, 14 out of 20; of the air cocci, 5 out of 18; and of the skin cocci, 5 out of 6, reduced nitrates to nitrites. It thus appears that the reduction of nitrates to ammonia is very common among members of the *Coccaceæ*, but that the reduction to nitrites only is variable and not characteristic of any one type.

The average amounts of gelatin liquefied after 30 days' growth at 20°C., are as follows: by the salivary cocci, 2.8 cc.; by the air forms, 1.9 cc.; and by those of the skin, 1.4 cc. Fifteen out of 20 of the salivary organisms, 15 out of 18 of the air forms, and 4 out of 6 of the skin cocci, liquefied gelatin. Summing up the results obtained from the experiments on gelatin liquefaction, it is to be noted that, in general, the salivary cocci liquefy gelatin more readily than do the air or skin forms, but aside from this it is apparent that there is nothing to warrant the use of gelatin as a differential medium.

The results of the experiments on vigor of surface growth on agar slopes at 20°C., and 37°C., are given in table II. While it may be said, in general,—from the results given in this table—that the salivary cocci grow somewhat more vigorously at 37°C. than at 20°C., the air forms better at 20°C. than at 37°C., and the skin organisms about equally well at the two temperatures, the differences are not sufficiently pronounced to impart to the factor of vigor of surface growth any marked value as a differential characteristic.

TABLE II

DATA ON THE VIGOR OF SURFACE GROWTH OF AIR, SALIVARY, AND DERMAL COCCI

Source of organism	Temperature of incubation	No. of cultures used	Growth characteristics					
			No growth	Very faint	Meager	Good	Abundant	Very heavy
Saliva	20°C.	20	—	3	7	10	—	—
Air	20°C.	18	1	—	—	4	7	6
Skin	20°C.	6	—	1	2	2	1	—
Saliva	37°C.	20	—	—	7	13	—	—
Air	37°C.	18	2	1	9	6	—	—
Skin	37°C.	6	—	1	—	4	1	—

In the following enumeration are listed the colors of the various pigments produced by the air, skin, and salivary cocci, the figure on the left having reference to the number in Ridgway corresponding to the particular pigment produced:

Salivary cocci

15″ d	Light pinkish cinnamon	3	
15″ c	Intermediate between light pinkish and pinkish cinnamon	4	
15″ b	Pinkish cinnamon	4	15
15″ a	Intermediate between pinkish cinnamon and cinnamon	3	
15″	Cinnamon	1	
21′ e	Intermediate between massicot and straw yellow	1	
23′ f	Naphthalene yellow	1	
19 f	Maize yellow	1	
19′ b	Mustard yellow	1	
	One gave too little growth for determination of the color.		

Air cocci

21′	f	Massicot yellow	4	16
21′	e	Intermediate between massicot and straw yellow	2	
21′	d	Straw yellow	1	
21′	b	Amber yellow	1	
19′	d	Naples yellow	1	
19′	b	Mustard yellow	4	
19′		Primuline yellow	1	
19	f	Maize yellow	1	
19	d	Buff yellow	1	
3′	b	Light Jasper red	1	

One form did not grow.

Skin cocci

19	f	Maize yellow	1	5
19	d	Buff yellow	1	
19	b	Apricot yellow	2	
21′	e	Intermediate between massicot and straw yellow	1	
		White	1	

At first glance the color of the pigments produced by the organisms would seem to furnish one mode of differentiation. In the majority of cases the salivary cocci produced cinnamon colored pigments, whereas pigments of a yellow color were usually produced by the air and skin forms. Closer inspection shows, however, that some of the salivary cocci, as well as the air forms, produce a maize yellow and a mustard yellow pigment; also that a maize yellow pigment and one intermediate between massicot and straw yellow are produced by representatives of both the salivary and skin cocci. It is apparent that these intergradations make the factor of pigment production largely inapplicable as a differential test.

In milk the salivary cocci with one exception produced acid and coagulated the medium, whereas none of the air forms and but one of the skin cocci gave this combined reaction. This attaches to milk considerable value as a differential medium. In the media containing the various organic substances

—sugars, etc., none of the coccus forms produced gas. All but one of the salivary cocci produced acid in the lactose medium, whereas none of the air cocci and but one of the skin forms deported themselves in this manner. This marks lactose broth as another medium of differential value.

The salivary cocci with but one exception produced acid in saccharose, the single exception being the organism which produced no acid in the lactose medium. Two air cocci and one skin form also produced acid in saccharose, but notwithstanding these exceptions, it appears that saccharose is a third valuable differential medium. In the mannite, salicin, inulin, sorbite, raffinose, and rhamnose broths none of the organisms produced acid, thus marking these organic substances as of no value in differentiating the types of cocci under investigation.

SUMMARY

In reviewing the preceding discussion of results we find three media, namely, lactose and saccharose broths, and milk, which are of value in differentiating the cocci most characteristic of saliva from those of the air and the skin. One of the salivary coccus forms did not produce acid in lactose and saccharose broths and formed neither acid nor clot in milk. This may have been, and probably was, an air or skin form. Among the air cocci are two which vary somewhat from the remaining air and skin forms in that they produce acid in saccharose broth. Neither of them, however, produces acid in lactose broth, nor acid or clot in milk and in these respects they differ markedly from the characteristic salivary forms. Of the skin cocci one gave the characteristic reactions of the salivary organisms, and it is not at all unlikely that this was a salivary coccus. In general, then, it appears that the organism most characteristic of saliva is a coccus form which produces acid in lactose and in saccharose broths, and acid and clot in milk.

FURTHER TESTS

To further test the validity of the reactions above referred to as furnishing a reliable means of differentiating between salivary cocci and those of other origin, two additional samples of saliva, from two different individuals, were examined,—

one from a middle aged white person (A), the other from a colored person (B).

The samples were collected and treated in a manner similar to that outlined in the early part of this paper. In the first case (A), transfers were made from all colonies on two plates, representing a dilution of one part saliva in ten billion. These subcultures, all of cocci, were numbered from 1 to 17 inclusive.

In the second case (B), transfers were made from 36 colonies which developed on one-third of a plate representing a dilution of one part saliva in ten billion. The entire series of cultures, numbered from 1 to 36 inclusive, although made from 36 colonies from a plate containing a total of 100 colonies, were found to be made up of coccus organisms. After being incubated in + 1 nutrient broth for 2 days at 37°C., each of the cultures from samples (A) and (B) was transferred to the three differential media,—lactose and saccharose broths, and milk. The results recorded in tables III and IV were observed after 3 days' incubation at 37°C. No gas was produced in any of the sugar media. A blank determination gave negative results throughout on the three media.

TABLE III

REACTIONS OF SALIVARY COCCI (A)

No. of culture	Lactose broth	Sacch. broth	Milk		No. of culture	Lactose broth	Sacch. broth	Milk		No. of culture	Lactose broth	Sacch. broth	Milk	
			Acid	Clot				Acid	Clot				Acid	Clot
1	+	+	+	+	7	+	+	+	+	13	+	+	+	+
2	+	+	+	+	8	+	+	+	+	14	+	+	+	+
3	+	+	+	+	9	+	+	+	+	15	+	+	+	+
4	O	O	O	O	10	+	+	+	+	16	+	+	+	+
5	+	+	+	+	11	+	+	+	+	17	+	+	+	+
6	+	+	+	+	12	+	+	+	+					

+indicates positive reaction. O indicates negative reaction.

From the above table it is evident that all but one of the coccus forms in series (A) produced acid in lactose and saccharose broths, and acid and consequent clotting in milk. The one exception was probably an air coccus.

TABLE IV

REACTIONS OF SALIVARY COCCI (B)

No. of culture	Lactose broth	Sacch. broth	Milk		No. of culture	Lactose broth	Sacch. broth	Milk		No. of culture	Lactose broth	Sacch. broth	Milk	
			Acid	Clot				Acid	Clot				Acid	Clot
1	+	+	+	+	13	+	+	+	+	25	+	+	+	+
2	O	+	O	O	14	+	+	+	+	26	+	+	+	+
3	+	+	+	+	15	+	+	+	+	27	+	+	+	+
4	+	+	+	+	16	+	+	+	+	28	+	+	+	+
5	+	+	+	+	17	+	+	+	+	29	+	+	+	+
6	+	+	+	+	18	O	+	O	O	30	+	+	+	+
7	+	+	+	+	19	+	+	+	+	31	+	+	+	+
8	+	+	+	+	20	+	+	+	+	32	+	+	+	+
9	O	+	O	O	21	O	+	O	O	33	+	+	+	+
10	+	+	+	+	22	+	+	+	+	34	+	+	+	+
11	+	+	+	+	23	+	+	+	+	35	+	+	+	+
12	+	+	+	+	24	+	+	+	+	36	+	+	+	+

+indicates positive reaction. O indicates negative reaction.

In series (B), 32 out of the 36 cocci reacted positively throughout on the three differential media. The remainder were positive with saccharose, but negative with lactose and milk, agreeing in this respect with the two air cocci to which reference has been made.

The reactions of the organisms from saliva (A) and (B)

further indicate that the production of acid in lactose and saccharose broths, and a similar production, together with clot, in milk, are characteristic reactions of the salivary cocci.

CONCLUSIONS

From the results of the preceding experiments it appears that a method applicable for the detection of the organisms characteristic of human saliva has been developed.

It must be acknowledged that the number of organisms examined is comparatively small, especially where those of the air and the skin are concerned. An absolute test of the validity of the adopted mode of identification would necessitate the examination of many hundreds of strains of cocci from numerous sources.

Nevertheless, the characteristic reactions of the salivary cocci examined seem to be sufficiently definite to warrant the assumption that the most characteristic salivary organism is a coccus form which produces acid in lactose and saccharose broths, and acid and clot in milk.

The Relation of the Most Characteristic Salivary Organism to the Pollution of Air

Having identified the most characteristic salivary organism, the next problem is to isolate it from the air. Its frequency of occurrence must also be determined, as this often serves as an index to the degree of pollution. The isolation of the organism and the determination of its frequency of occurrence can be accomplished simultaneously.

Then come the problems (1) of devising an air-collecting apparatus suitable for all occasions, and (2) of determining the quantity of air to be examined and the terms by which the sanitary quality of the air shall be expressed.

In searching for a means of expressing the sanitary quality of air, let us consider the manner in which this is accomplished in drinking water. Authorities differ markedly on this subject. Shall a water be considered safe or unsafe for drinking purposes if *B. coli* is present in a 100 cc. sample, or shall its presence or absence in 10 cc. or 1 cc. samples be taken as the basis for the

classification? In lieu of a definite standard let us assume the following table[1]:

TABLE V

PRESUMPTIVE TEST FOR *B. COLI* IN WATER

Sanitary quality	cc. 0.01	cc. 0.1	cc. 1.0	cc. 10.0	cc. 100
Safe	O	O	O	O	+
Reasonably safe	O	O	O	+	+
Questionable	O	O	+	+	+
Probably unsafe	O	+	+	+	+
Unsafe	+	+	+	+	+

+ indicates positive presumptive test for *B. coli*.

We shall now endeavor to prepare a similar table for the purity of air, expressed in the number of salivary cocci present in given volumes. In the normal life processes, the volume of air inhaled is obviously much greater than the volume of water consumed, and this fact must be taken into consideration in establishing a criterion for the bacteriological examination of air. It has been estimated that the tidal air, i.e., the air taken in with each inspiration and given out with each expiration, amounts, in a normal adult when at rest, to one-half liter. Assuming the average frequency of respiration to be 15 per minute, the amount of air inhaled in one minute is $7\frac{1}{2}$ liters, in one hour, 450 liters, and in one day, at least 10,000 liters. Taking the average amount of unboiled water drunk in a day as 2 liters, it would appear that 5,000 times as much air as water is required daily. Hence, the following table, based on table v, may be used to express the sanitary quality of air:

[1] Whipple, G. C. On the practical value of presumptive tests for *B. coli* in water. Techn. Quart. **16**:18 e. m. 31. 1903.

TABLE VI

TEST FOR CHARACTERISTIC SALIVARY COCCI IN AIR

Sanitary quality	cc. 50	cc. 500	cc. 5,000	cc. 50,000	cc. 500,000
Safe	0	0	0	0	+
Reasonably safe	0	0	0	+	+
Questionable	0	0	+	+	+
Probably unsafe	0	+	+	+	+
Unsafe	+	+	+	+	+

+ indicates positive reaction in the three differential media adopted.

APPARATUS AND TECHNIQUE

As it was the intention to collect samples of air in places other than the laboratory, a portable apparatus was necessary. As devised, it consists essentially of a sand filter, a support for same with an attachment for alternately opening and closing the exhaust and suction, and a bulb, having a capacity of 16 oz., with the required amount of rubber tubing. (See plate 2.) The sand filter is of the standard type. It consists of a glass tube 100 mm. long and 10 mm. in diameter, fitted with a one-hole rubber stopper, through which passes a piece of 6 mm. glass tubing. This stopper, with its tubing, forms the support for a circular disc of bolting cloth with a 10 mm. layer of very fine clean quartz sand that passes through a 100, and is retained on a 140 mesh sieve.

The support consists of a rectangular piece of wood 12 x 1 x $\frac{3}{4}$ inches, fitted with a double pinch cock arrangement. Clamps for holding the filter in position are also provided. The rubber bulb is connected to the apparatus in such a manner that when pressure is applied to the former and the pinch cock opened, the air contained in the bulb is expelled through the exhaust without disturbing the sand in the filter in any way. This operation occupies but a few seconds of time. Upon releasing the pinch cock, and immediately thereafter the bulb, the air is drawn through the sand.

The volume of air exhausted from the bulb at each pressure was determined as follows: The bulb, filled with water, was weighed. Pressure was then applied, forcing out the water, after which the bulb was again weighed. The difference in weight in grams is approximately the volume of air in cc. exhausted by a similar pressure. In the calibrations the results varied but slightly. By placing the fingers on the bulb in a certain fixed position each time, it was found that the bulb could be made to deliver 300 cc. of air at each exhaustion and, consequently, to receive 300 cc. of air at each release of pressure. It was, of course, necessary to have all joints air-tight, this being accomplished by making all connections with rubber tubing and glass and using plenty of overlap.

The sand filter, after being plugged at both ends with cotton, was sterilized for 30 minutes at 15 pounds pressure. The rubber stopper support was allowed to fit very loosely into the tube during sterilization in order to prevent setting of the rubber. After the apparatus was removed from the autoclav, the stopper was immediately fitted in tightly, thus rendering the connection air-tight. The sand filter was always used within 24 hours after sterilization. It was connected to the support as shown in plate 2.

When operated in public buildings or conveyances, the support, with the filter, was wrapped in stiff paper in such a manner as to permit of the easy operation of the pinch cock and exhaust. The apparatus thus wrapped was held in the left hand and from it heavy rubber tubing passed down the left coat sleeve and then diagonally across to the right coat pocket where it was connected to the bulb. This rendered the whole apparatus inconspicuous. The bulb was operated with the right hand, the pinch cock with the left. A test tube with a sterile cotton plug was always carried, the latter being used to replace the plug which was removed from the intake of the filter at the beginning of the experiment.

The plating was always carried out within 30 minutes after the sample was obtained. The sand from the filter was carefully poured into a 100 cc. flask containing 15 cc. of sterile distilled water. The bolting cloth, which had a tendency to stick to the rubber stopper, was removed with sterile forceps and introduced

into the flask. The contents of the flask were thoroughly shaken and aliquot portions, as shown in table IX, were plated with 10 cc. of +1 nutrient agar. In plating, the introduction of much sand was avoided in the following manner: The end of the pipette was held immediately above the bottom of the flask while the liquid was being drawn up to a point slightly above the gradua-tion mark. After a few seconds, enough of the sandy liquid was allowed to run back into the flask to leave the water just at the mark. During this short interim a large proportion of the sand settled in the tip of the pipette and was returned to the flask as the liquid was lowered to the mark. Blanks were plated several times during the course of the experiments, but no growth developed in any case.

The plates were in all cases incubated for 4 days at 37°C., after which the number of bacterial colonies present in each was determined. Finally, all, or a representative number, of the colonies were examined for the presence of coccus forms. (See table IX.)

The coccus colonies developing on agar are, as a rule, very small and often grow in the deeper strata of the medium. This renders the transfer difficult especially when two are to be made from the same colony—one for the stained preparation and one for the agar slope to be used as a stock culture. The difficulty was partially obviated by subculturing (from all the colonies in certain selected plates) to agar slopes, and incubating the latter at 37°C. After several days an examination served to eliminate the bacilli and moulds, leaving only the coccus cultures which were later examined for the presence of the sali-vary forms. In this examination the three differential media described above were used.

SOURCES OF SAMPLES

As the investigation in hand seeks to discover a relation be-tween the presence of a characteristic salivary organism and the pollution of air, it was thought best to collect the samples of air under normal conditions, i.e., conditions which are met with in every-day life.

Public conveyances, on account of their usually crowded condi-tion and frequently inefficient ventilation, suggested themselves

as favorable places for tests. Hence, a local street car was chosen as a source for air samples. The often poorly ventilated but well filled motion picture theatres furnished another supposedly promising sampling place. The third locality chosen as a source for air samples was a local 5 and 10 cent store. It was thought that this would furnish an ideal source of contaminated air because of the large crowds of people who are continually voicing their sentiments and desires. In order to determine whether or not the salivary organism is present in an atmosphere which is not in immediate contact with human beings, and which is open to the ventilation of nature,the fourth sample was taken from the open air.

DISCUSSION OF THE EXPERIMENTS

The experiments in table IX are arranged according to the dates on which the tests were made. But for convenience in this discussion the experiments will be taken up according to the source of the samples.

Experiment 1.—This experiment was carried out primarily to test the apparatus. The air sample was taken in a laboratory on the second floor of an old building. There were usually at least two people present in the room, and practically no ventilation was provided, the doors and windows being constantly closed. The apparatus used differed from that used in the remaining experiments in that two sand filters were used in tandem instead of the usual one. During the 15 minutes of operation, 7,800 cc. of air were drawn through the sand of both filters at the rate of 520 cc. per minute.

The sand of the first filter was introduced into 15 cc., that of the second into 6 cc. of sterile distilled water. Quantities of both solutions were plated with the following results:

TABLE VII

Filter number	Plate number	Quantity plated	Total no. of colonies	Coccus colonies
1	1	1 cc.	2	0
1	2	1 cc.	2	2
1	3	2 cc.	4	2
2	4	5 cc.	0	0

The reactions of the cocci isolated from the air in the first filter showed that there was one salivary coccus form present. The remaining three gave negative reactions on the three differential media. It should be noted that out of the 15 cc. of solution from the first filter, only 4 cc. were plated. Eight organisms were present in the quantity examined, making a total of 30 in the entire solution. One characteristic salivary coccus form developed in the portion examined, making, according to the law of averages, a total of 4 in the entire solution. The total volume of air examined being 7,800 cc., the frequency of occurrence of the salivary coccus is 1 in 1,950. According to table VI, the sanitary quality of the air of the room was "probably unsafe" at the particular time at which the sample was taken.

EXPERIMENTS 3, 5. 6, 8, 10

These experiments were carried out in a local street car. The same car line was chosen for all of the experiments in order to eliminate as many variables as possible, such as construction of car, capacity, rate of locomotion, etc. The car was of the ordinary "pay-as-you-enter" type now in use in St. Louis. It had a seating capacity of about 44 people, and could accommodate approximately 40 more standing indoors. The air space in the car in question was about 2,500 cubic feet, or approximately 30 cubic feet for each passenger when the car was filled to its capacity.

As the samples were taken at a time when the outside temperature would not permit the windows to be open, the question of ventilation was carefully studied. As is usually the case, the transoms were tightly closed, and only when the front and rear doors of the car were open at the same time was there an opportunity for a complete renewal of the air. This never happens when the car is in motion, and there is probably never a complete renewal of air unless a strong wind is blowing, thus causing a draught when the car is at a standstill, with both doors open. This particular car was provided with four vents in the roof which could be opened or closed at will. In several of the experiments some of the vents were open; in others, all were closed.

The degree of pollution of the atmosphere in such a car de-

pends, of course, on the amount of coughing, sneezing, speaking, etc., of its occupants. A car may be very crowded but if no coughing, etc., is going on, there will, theoretically, be no pollution of the atmosphere from saliva. Again, if there is much talking, etc., among those present, the atmosphere may be greatly polluted by the dissemination of particles of saliva from the mouth.

The samples were always taken in the early morning between the hours of six and seven, when the majority of the laboring class are on their way to work. The tendency of the passengers at this time of the day is to be quiet, as the morning paper is of absorbing interest to a majority. The samples of air were taken in the center of the car, the opening of the apparatus being about 4 feet from the floor level. In these experiments the apparatus described above was used. In all cases 10,800 cc. of air were drawn through the sand filter at the rate of 900 cc. per minute. The sand was introduced into 15 cc. of sterile distilled water and plated as shown in table IX.

The experiments carried out in street cars will now be taken up in order and the results discussed. If it can be shown that the characteristic salivary organism is present in the air of these cars in sufficient quantity, and if it can later be proved that this salivary organism is not present in the open air, it follows that the atmosphere in these cars is being polluted by the dissemination of particles of saliva from the mouth.

Experiment 3.—While the air sample was being taken for this experiment, 44 people were seated in the car, but none were standing. Out of the 20 colonies appearing on the plate (see table IX), 9 were of bacilli and 11 of coccus forms. Inoculated into the 3 differential media, 8 of the latter reacted negatively in all three media, 1 negatively on lactose and milk, but positively on saccharose, and 2 gave positive reactions in all three media.

It will be recalled that mention has been made of several organisms, both among the salivary and air cocci, which gave a positive reaction with saccharose, but reacted negatively with lactose and milk. The one above referred to as reacting in this manner is probably one of these unidentified coccus forms which seem to be present in both saliva and air. Out of

the 11 cocci present, therefore, two were of the characteristic salivary type, and as only one-third of the sample was plated, a total of 6 may have been present in the entire volume of air examined, or a frequency of occurrence of 1 in 1,800. According to table VI, the sanitary quality of the air was "probably unsafe."

Experiment 5.—During the sampling process for this experiment, 44 persons were seated and approximately 30 standing. Of the 26 colonies which developed on plate 5 (see table IX), 14 were of bacilli, 2 of streptothrix, and 10 of cocci. When transferred to the three differential media, all of the latter gave negative reactions, indicating that the air in the car at the time of this experiment was "safe."

Experiment 6.—At the time of sampling, 44 persons were seated and 30 were standing. On account of the large number of colonies present, only a representative sector of plate 1—comprising one-twelfth of the total area—was examined (see table IX). On this area 21 colonies were counted, 5 bacillus and 16 coccus. On the three differential media, 4 of the latter gave negative reactions throughout, 6 were negative on lactose and milk but positive on saccharose, and 6 gave positive reactions on all three media. It follows that 6 salivary cocci were isolated from one-twelfth of the plate, making a total of 72 from the entire plate, or of 1,080 from the total volume of sand solution,—a frequency of occurrence of 1 in 10. According to table VI, the air in the car at the time of the experiment was "unsafe."

Experiment 8.—The number of persons seated and standing was the same as in experiment 6. On the plate examined (see table IX), 34 colonies developed—15 bacillus and 19 coccus. On the three differential media the coccus forms reacted as follows: Twelve gave negative reactions throughout, 6 were negative on lactose and milk but positive on saccharose, and 1 was negative on lactose and saccharose but positive on milk. The last form was found, after again staining with gentian violet and examining under the microscope, to be a short bacillus. It is to be noted that 6 organisms of the unidentified coccus type were again present. No characteristic salivary cocci were present, thereby marking the air of this particular car as "safe" at the time of the experiment.

Experiment 10.—During this experiment, 44 persons were seated and 15 standing. It was noted that one transom was open. The plate examined (see table IX) gave a total of 12 colonies, of which 5 were of bacilli and 7 of cocci. Of the latter, 6 reacted negatively on all three of the differential media, whereas 1 gave a positive reaction throughout. This makes the frequency of occurrence of the characteristic salivary coccus form 1 in 3,600, and, according to table VI, marks the air in this car as "questionable" at the time of the experiment.

Summarizing the car experiments, it is to be noted that in three out of five cases the characteristic salivary coccus form was isolated, and in such quantity as to mark the air of one "unsafe," that of another "probably unsafe," and of a third "questionable."

EXPERIMENTS 9 AND 11

These experiments were carried out in a local vaudeville house. The construction of the building appeared modern in every respect. The lower floor had a seating capacity of about 2,000, while the balcony accommodated approximately 1,000 people. The house was filled with spectators on the occasions when the samples were taken. Upon inquiry, after the surprisingly good results given below were obtained, it was found that the building was well ventilated by one of the modern appliances for this purpose, whereby the volume of air in the building (about 90,000 cubic feet) was being renewed to a greater or less extent every seven-tenths of a minute. For the collection of the air samples, the same apparatus was used as in the street car experiments, 10,800 cc. of air being drawn through the sand filter at the rate of 900 cc. per minute. The sand was introduced into 15 cc. of sterile distilled water and platings were made as indicated in table IX.

Experiment 9.—The air sample was obtained near the center of the lower floor of the building about 60 feet from the stage. The entire lower floor was packed, and in addition about 100 or more persons were standing in the rear. On the plate examined, a total of 14 colonies developed,—3 mold, 9 bacillus and 2 coccus. Molds were very abundant on the other plates. One of the coccus forms reacted negatively on lactose and milk but positively on saccharose, whereas the other gave negative

reactions on all three differential media. The presence of the single unidentified coccus is again noted. No salivary coccus forms were isolated, from which fact it appears that the air in the particular location from which the sample was taken was "safe."

Experiment 11.—This sample was taken on the balcony of the building, about 10 feet from the rear wall. Every seat was occupied. As indicated in table IX, two plates were examined. On the first, 7 colonies developed—3 streptothrix, 3 bacillus, and 1 coccus. On the second plate 3 colonies appeared, all of which were of bacilli. The reaction of the coccus was negative on the three differential media, thereby indicating that the sample of air taken was free from salivary coccus forms and therefore "safe."

In summing up the results of the experiments carried out in the vaudeville house it is to be noted that in both cases no salivary coccus forms were found. Table IX further shows that the total number of organisms found per unit volume of air was smaller than in the street car experiments.

EXPERIMENTS 4 AND 7

These samples were obtained in the basement of a local 5 and 10 cent store. The ceiling was rather low, being only about 9 feet from the floor level, the entire basement having a volume of about 72,000 cubic feet. The samples in these experiments were taken in the midst of a crowd gathered to listen to a singer advertising songs. Little attention was given to the matter of ventilation until after the results of the experiments were obtained. Subsequently, however, investigation revealed the fact that ample provision had been made for ventilation. Transoms at the level of the sidewalk provide openings to the outside; along the inside wall and near the ceiling are revolving fans about 20 feet apart. These keep the air in circulation until it is drawn out by a suction fan situated in one corner, about 2 feet from the ceiling. The same sampling apparatus was used as in the preceding experiments. As before, a total of 10,800 cc. of air was drawn through the sand filter in each sampling at the rate of 900 cc. per minute. The samples were plated as shown in table IX.

Experiment 4.—The air sample for this experiment was taken

in the midst of a crowd of about 100 people in front of a counter. On the plate examined, a total of 36 colonies developed—16 bacillus and 20 coccus. Of the latter, 18 gave negative reactions throughout on the three differential media, and 2 reacted negatively on saccharose and milk but positively on lactose, the latter sugar being fermented. No salivary coccus forms were isolated, indicating that the air in the basement at the time of the experiment was "safe."

Experiment 7.—This air sample was taken under practically the same conditions as in the previous experiment except that only about 50 people were in the crowd. The plate examined contained 2 streptothrix, 13 bacillus, and 8 coccus colonies. All of the cocci gave negative reactions throughout on the three differential media. No salivary coccus forms were found, which fact leads again to the conclusion that the sanitary quality of the air during the experiment was "safe."

EXPERIMENTS 2, 12, 13, 14

These experiments were performed outdoors. The air sample for experiment 2 was collected in a railroad switch yard at a time when there was no traffic. The samples for experiments 12, 13, and 14 were collected in the immediate vicinity of large storage basins belonging to the local water works and located 300 or 400 feet from the bank of the Mississippi River. The apparatus used was the same as that employed in the previous experiments.

Experiment 2.—The outdoor temperature was 29°F., and while the air sample was being taken it was snowing. A total of 22,500 cc. of air was drawn through the sand filter at the rate of 750 cc. per minute—the operation extending over a period of 30 minutes. Samples were plated as shown in table IX. Of the 3 plates examined, plate 1 yielded 2 bacillus colonies; plate 2, 1 streptothrix, 1 bacillus, and 6 coccus colonies; and plate 4, 2 mold, 1 streptothrix, 1 bacillus, and 2 coccus colonies. All of the coccus forms were grown on the three differential media, 7 giving negative reactions throughout, while 1 reacted positively on saccharose and negatively on lactose and milk. The latter organism is one of the unidentified coccus forms previously referred to. No characteristic salivary cocci were found, in-

dicating that the sanitary quality of the air examined was "safe."

Experiment 12.—At the time the air sample was being taken, a slight drizzling rain was falling, accompanied by considerable wind and a temperature of 45°F. Prior to that time it had been raining continuously for about 24 hours. A total of 10,800 cc. of air was drawn through the sand filter at the rate of 900 cc. per minute, the apparatus meanwhile being held about 5 feet above the ground level. The sand of the filter was introduced into 15 cc. of sterile distilled water, from which platings were made. Table VIII gives the details of the experiment, together with the results obtained.

TABLE VIII

Plate number	Quantity plated	Total no. of colonies	No. of bacteria and molds	Coccus colonies	No. of salivary cocci
1	1 cc.	9	2	7	7
2	1 cc.	3	3	0	0
3	5 cc.	0	0	0	0
4	5 cc.	6	4	2	0

Attention should be called to the fact that on plate 1, in which only 1 cc. of the solution was used, 9 colonies developed—7 coccus and 2 bacillus—, while on plate 4, in which 5 cc. of the solution were used, only 6 colonies appeared,—2 coccus and 4 bacillus. Furthermore, the 7 colonies in plate 1 proved to be of salivary cocci, whereas none of these organisms were present among the cocci of plate 5. These results unquestionably indicatelocal contamination. It is difficult to say just where the contamination took place. Obviously it did not occur during the collection of the sample or even during the mixing of the sand solution; for had this been the case all of the plates should have shown salivary cocci, and the greater number should have occurred on those plates in which larger quantities of the solution were plated. In all probability plate 1 was locally contaminated.

Experiment 13.—While the sample of air was being taken for this experiment, the temperature was 63°F., a light breeze was blowing, and the sky was very cloudy although no rain had

fallen during the preceding 18 hours. A total of 10,800 cc. of air was drawn through the apparatus at the rate of 830 cc. per minute. Samples of the sand solution were plated as shown in table IX. It is to be noted that in those plates containing 1 cc. of the solution no colonies developed, whereas in those containing 5 cc., 1 bacterial colony appeared in each. Attention is called to the consistent results in this experiment to emphasize the fact that the inconsistencies in experiment 12 are due to local contamination. No salivary cocci were found.

Experiment 14.—The air sample for this experiment was taken on a bright, clear day, with a rather strong wind blowing and a temperature of 55°F. A total of 10,800 cc. of air was drawn through the sand filter at the rate of 1,080 cc. per minute. Samples of the sand solution were plated as shown in table IX. Of the 3 coccus forms, 2 gave negative reactions on all three differential media, whereas 1 was positive on saccharose and negative on lactose and milk. The latter will be recognized as one of the unidentified coccus forms. No salivary cocci were isolated.

Summarizing the open air experiments, it is to be noted that, barring the locally contaminated plate 1 in experiment 12, the characteristic salivary coccus form was not isolated; furthermore, that the total number of organisms in the open air is comparatively low.

Summary and Conclusions

Examining the entire series of experiments it appears that in the majority of cases where ventilation was obviously inadequate, the characterisitic salivary coccus form was isolated. On the other hand, the form could in no case be found where ample artificial or natural ventilation existed.

It has been shown that the most characteristic salivary organism can be differentiated and identified; also, that this characteristic organism can be isolated from the air.

In the experiment carried on in one of the street cars in which there were many passengers, the characteristic salivary coccus form was found to be present in such quantities as to indicate that the air in this car was "unsafe." It was later shown that

TABLE IX

DATA ON THE COLLECTION AND EXAMINATION OF AIR SAMPLES

No. of experiment		1	2	3	4	5	6	7
Date of collection		2/12/13	2/22/13	3/15/13	3/15/13	3/19/13	3/22/13	3/22/13
Sampling place		Lab.	Outdoors	Street car	5 and 10c. store	Street car	Street car	5 and 10c store
Temperature (°F.)	Outdoors	60	29	32	36	50	23	50
	Sampling pl.	80	29	45	70	48	45	70
Weather conditions		Sunshine	Snowing	Windy snowing	Windy snowing	Sunshine	Sunshine	Sunshine
Approx. no. of persons	Sitting	0	0	44	1	44	44	1
	Standing	2	0	0	100	30	30	50
Approx. volume of sampling pl. (cu. ft.)		3000		2500	72000	2500	2500	72000
Volume of air exam. (cc.)		7800	22500	10800	10800	10800	10800	10800
Rate of filtration (cc. per min.)		520	750	900	900	900	900	900
No. of organisms in following quantities of sand solution plated	Pl. I. (1 cc.)	2	2	4	30	9	250	4
	Pl. II. (1 cc.)	2	8	3	36	6	250	3
	Pl. III. (2 cc.)	4	0					
	Pl. IV (5 cc.)		6	12	230	25	Too numerous to count.	Spreader
	Pl. V. (5 cc.)			20	200	26	Too numerous to count.	23
Plates examined		I., II. and III.	I., II. and IV.	V.	II.	V.	1/12 of I.	V.
Total col. on plates exam.		8	16	20	36	26	21 on 1/12 of I.	23
No. of bacilli, molds, etc.		4	8	9	16	16	5 on 1/12 of I.	15
No. of cocci		4	8	11	20	10	16 on 1/12 of I.	8
No. of salivary cocci		1	0	2	0	0	6 on 1/12 of I.	0
Frequency of occurrence		1 in 1950	0	1 in 1800	0	0	1 in 10	0
Sanitary quality		Probably unsafe	Safe	Probably unsafe	Safe	Safe	Unsafe	Safe
No. of org. in total vol. of air		30	42	50	570	95	3750	58

TABLE IX (*Continued*)

DATA ON THE COLLECTION AND EXAMINATION OF AIR SAMPLES

No. of experiment		8	9	10	11	12	13	14
Date of collection		3/29/ 3	3/29/13	4/1/13	4/1/13	4/8/13	4/9/13	4/10/13
Sampling place		Street car	Picture show	Street car	Picture show	Outdoors	Outdoors	Outdoors
Temperature (°F.)	Outdoors	41	59	50	77	45	63	55
	Sampling pl.	55	70	63	82	45	63	55
Weather conditions		Sunshine	Cloudy	Sunshine	Sunshine	Rain, very windy	Cloudy	Sunshine, very windy
Approx. no. of persons	Sitting	44	3000	44	3000	0	0	0
	Standing	30	100	15	300	0	0	0
Approx. volume of sampling pl. (cu. ft.)		2500	90000	2500	90000			
Volume of air exam. (cc.)		10800	10800	10800	10800	10800	10800	10800
Rate of filtration (cc. per min.)		900	900	900	900	900	830	1080
No. of organisms in following quantities of sand solution plated	Pl. I. (1 cc.)	34	3	4	7	9	0	2
	Pl. II. (1 cc.)	18	2	2	3	3	0	2
	Pl. III. (2 cc.)	24						
	Pl. IV. (5 cc.)	Spreading mold	14	Spreader	Spreader	0	1	3
	Pl. V. (5 cc.)		8	12	35*	6	1	1
Plates examined		I.	IV.	V.	I. and II.	I., II. and V.	IV. and V.	I.,II.,IV., and V.
Total col. on plates exam.		34	14	12	10	18	2	8
No. of bacilli, molds, etc.		15	12	5	9	9	2	5
No. of cocci		19	2	7	1	9	0	3
No. of salivary cocci		0	0	1	0	7	0	0
Frequency of occurrence		0	0	1 in 3600	0	1 in 1235	0	0
Sanitary quality		Safe	Safe	Question-able	Safe	†	Safe	Safe
No. of org. in total vol. of air		320	35	42	85	50	2	18

* Abundance of molds.
† Local contamination.

the salivary coccus form could not be found in the open air devoid of the immediate presence of human beings.

It thus appears that the presence of the salivary coccus form in air indicates the presence of man; furthermore, it indicates the pollution of air by particles of mucus from the mouth.

Flügge [1] and his school have shown that pathogenic organisms may be transmitted into the air, and other workers [2] have shown that the tubercle organism is capable of being carried by even such feeble air currents as ordinarily exist in dwellings.

The tubercle organism, as well as the characteristic salivary organism, is present in the saliva of tubercular patients. If, therefore, this salivary organism can be isolated from the air by means of the filter used in the above experiments, does it not follow that the tubercle organism could be isolated in a similar way? Since our manner of breathing is comparable to the operation of the apparatus used, it follows that the tubercle organism may be inhaled by man.

It thus appears that the presence in the air of the most characteristic salivary organism is an index of the possible access of pathogenic organisms to the atmosphere.

In conclusion, the writer wishes to express his thanks to Dr. Geo. T. Moore, for valuable suggestions and numerous courtesies extended during the progress of the work; to Dr. J. R. Schramm, for suggestions, and aid in the preparation of the manuscript; and to Mr. Wilson F. Monfort, Chemist of the City of St. Louis Water Department, for advice given and opportunities provided for the collection and examination of the samples.

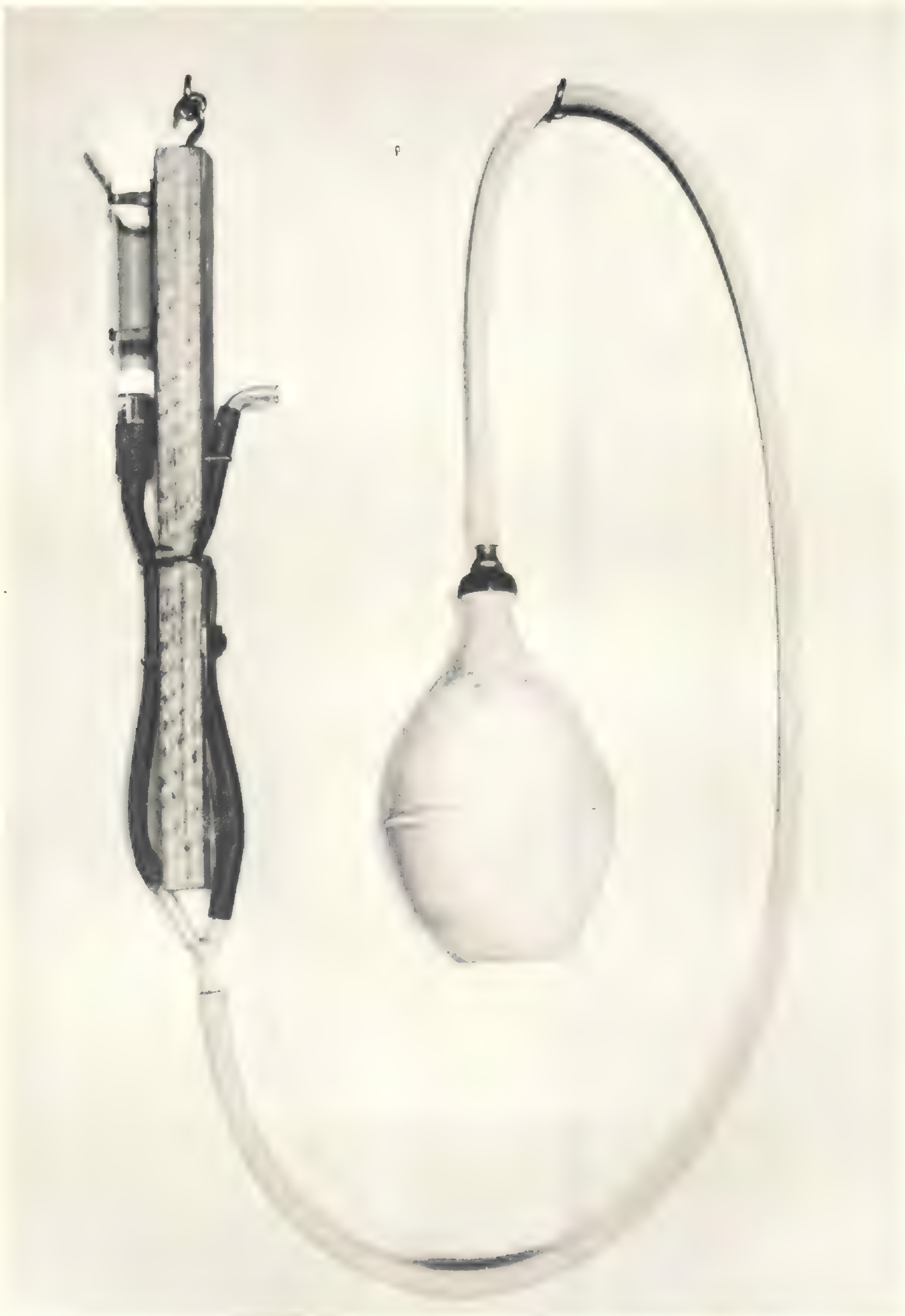
Explanation of Plate

PLATE 2

Air-sampling apparatus showing support, sand filter, pinch cock, exhaust and suction tubes, and pressure bulb.

[1] Gordon, M. H. *loc. cit.*

[2] Kolle and Wassermann, Handbuch der pathogenen Mikroorganismen 1: 169.



NOLTE—SALIVARY ORGANISMS AND AIR POLLUTION

THE POLYPORACEÆ OF OHIO

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Introduction

The *Polyporaceæ*, or "pore fungi," constitute a relatively small family of the *Basidiomycetes*, characterized by having the spores borne on the interior surfaces of tubes or pores which make up the hymenium of the fungus. In its most comprehensive sense the family embraces the two subfamilies *Boleteæ* and *Polyporeæ*, including also such aberrant genera as *Merulius*, *Porothelium*, *Solenia*, etc. More often the *Boleteæ* are made a separate family, the *Boletaceæ*, usually distinguished from the true *Polyporaceæ* by the more fleshy nature of the plant and by the fact that the pores rather easily separate in a smooth layer from the flesh of the pileus. The true *Polyporaceæ*, on the other hand are more commonly leathery, corky, or woody in texture, and only in rare cases are the tubes separable from the context. More recently Dr. Murrill, who has monographed the North American species of the family for the North American Flora—now being issued by the New York Botanical Garden—, has still further limited the family so as to exclude not only the genera referred to above, but also certain of the true polypores which possess a more or less gelatinous or waxy hymenium. For the reception of certain of these forms he has erected the family *Xylophagaceæ*.

C. G. Lloyd has published monographic papers on certain of the sections of the family, using for the most part as the generic names, the sectional names given by Fries. Within the past year a third system of classification has been proposed by Miss Ames, of Cornell University, who divides the family into groups on the character of the context, and these groups are separated into genera on the form of the fruit body, surface modifications, spore characters, etc. Various workers in Europe have at-

tempted to revise the genera of the *Polyporaceæ* but none of these classifications have been generally adopted by mycologists.

The family is here taken to include the following genera: *Polyporus* (including *Polystictus*), *Fomes*, *Trametes*, *Dædalea*, *Lenzites*, *Cyclomyces*, *Favolus*, *Glœoporus*, *Merulius*, and *Irpex*. Distributed among these genera are practically one hundred species found within the state. Of these, 78 have been collected by the writer,4 others have been sent in by correspondents, and examination has been made of collections of 5 other species taken within the state and preserved either at the Lloyd Museum at Cincinnati, or in the herbarium of the New York Botanical Garden. Of the remaining 12 species some are known only from the records left by Morgan, Lea, Montagne, Berkeley, and Kellerman, others are admitted because there is every reason to believe that they will be found within the state since they are known to have been collected in nearby counties of adjoining states.

The resupinate *Polyporaceæ*, usually included in the genus *Poria*, have been omitted from this paper. Very little is known in this country concerning these forms and very few authentic specimens were available for study and comparison. Most of the species that have been reported from this country have been based on scarcely more than a guess, and it is impossible for the amateur mycologist to determine his material from the confused and often fragmentary account that has been written. Until the genus has been thoroughly studied by a competent mycologist, only added confusion would result from anything more than a reference to it in this paper.

In the preparation of the keys, relationships, both of genera and species, have been entirely ignored, the aim being to produce a usable key rather than to exhibit relationships. The writer believes that the color of the context is one of the most constant of the gross characters of these plants, and the genera are divided into sections on that basis. The presence or absence of a stipe, the duration of the plant, the hymenial configuration, the surface markings of the pileus, etc., are brought into the key in an order which the writer believes corresponds to their relative importance as specific distinguishing characters. Spore characters, especially spore colors, are not used in the separation

of the genera, and in the separation of the species only where experience has shown that the spores are always easily obtained. In many cases it is impossible to obtain spores, especially if they be uncolored, from the hymenium of dried plants. However, when plants are taken in the fresh condition it is usually a simple matter to obtain them by leaving the fungus over night in a moist atmosphere and allowing the spores to fall upon a glass slide. Spores of the perennial woody forms may often be obtained by this method when an examination of the same material in the dried state does not reveal their presence. In this paper spore measurements have been freely taken from other publications, both European and American. This was done in order that the descriptions might be made more comparable. Due credit is given to the author in every case where this was done.

An effort has been made to make the descriptions exactly comparable one with another. For this purpose a definite sequence of presentation has been arranged for the different characters and this order preserved in all but a few instances in which entire descriptions were taken from the original sources. In the comments following each species the characteristic specific distinctions are pointed out and references are made to illustrations of one sort or another that give a good idea of the plant as the writer understands it. Practically all of these references are to papers published in this country. The writer has had access to all of the important publications on the family, both European and American. Most of the European writings are not available to a large part of those students for whom this paper is intended and it was believed that a careful selection of citations to the illustrations published in this country would be of more value than citations to the less known and often inaccessible European publications. Those who are in a position to look up additional references will have access as well to volumes 19 and 20 of Saccardo's 'Sylloge Fungorum,' where an exhaustive index to illustrations will be found.

It is believed that there can be no question of the need of a paper worked out along the above indicated lines. No such publication exists for any state in the Union and the only aids that students have had in determining their collections have

been either the incomplete "mushroom" books or such extensive works as 'Sylloge Fungorum' and in more recent years the monograph presented in the 'North American Flora.'

In the matter of citation and nomenclature an attempt has been made to follow the rules and recommendations of the International Botanical Congress at Brussels. Since there has been little opportunity to compare specimens of our plants with those of Europe or with type specimens, the procedure in the matter of synonymy has been very conservative. The only names cited as synonyms are those of which the writer has a personal knowledge gained from the examination of authentic material, usually species described from Ohio. Where there has been a doubt as to the identity of a plant in this country with that of one in the old world the procedure has been to use the name under which it has been described or known in this country.

The first and therefore the most complete set of specimens is in the herbarium of the writer; a set of all of the more common forms is in the herbarium of Dr. Bruce Fink, of Miami University, at Oxford, Ohio; a partial set is in the state herbarium, at Columbus; and a large number of species, sent to Dr. Murrill for determination and verification, are in the herbarium of the New York Botanical Garden.

The writer is under deep obligations to the following persons in various ways: First of all to Dr. Bruce Fink, under whose direction the work was begun, whose aid, criticism, and advice has made this publication possible; to Dr. W. A. Murrill, of the New York Botanical Garden, for many kindnesses in verifying and determining specimens sent to him, and for the privilege of studying the specimens in the herbarium at that place; to Mr. C. G. Lloyd, of Cincinnati, for the privilege of working in the Lloyd Library and Museum and for determinations of specimens; to Rev. G. Bresadola, of Trient, Tyrol, for determination of specimens; to Dr. E. A. Burt, of the Missouri Botanical Garden, for access to his herbarium and for suggestions as to the final form of the paper; and to all who have aided in the work by sending specimens and in various other ways.

It is hoped that the paper will be found useful not only to Ohio students but in the neighboring states of the Great Lakes

region and in the Ohio valley as well. It is with this idea in mind that the paper has been prepared.

Key to the Genera.

Sporophore entirely resupinate; pileus none.....*Poria*[1]
Sporophore sessile or stipitate, sometimes effused-reflexed but not normally entirely resupinate..... 1
1. Hymenium composed of concentric lamellæ; pileus stipitate..*Cyclomyces p.* 147
1. Hymenium not composed of concentric lamellæ; pileus sessile or stipitate... 2
2. Hymenium not distinctly poroid, the pores reduced to shallow pits separated by narrow ridges or reticulations.....*Merulius p.* 150
2. Hymenium distinctly poroid, irpiciform, dædaloid or lamellate, but not pitted..... 3
3. Hymenium more or less waxy or gelatinous, the layers of tubes separating smoothly from the context in fresh specimens or when moistened; pileus sessile, thin and flexible.....*Glæoporus p.* 149
3. Hymenium not at all waxy or gelatinous and not separating smoothly from the context; pileus sessile or stipitate..... 4
4. Hymenium either dædaloid, labyrinthiform or lamellate, at least in part 5
4. Hymenium poroid or sometimes broken up into teeth..... 9
5. Context white..... 6
5. Context brown..... 8
6. Pileus minutely velvety to glabrous; context more than 1 mm. thick *Dædalea p.* 143
6. Pileus hirsute to villous; context 1 mm. or less thick..... 7
7. Hymenium lamellate, at least in part.....*Lenzites p.* 145
7. Hymenium dædaloid but never lamellate .....*Dædalea p.* 143
8. Plants woody and perennial, more than 1 cm. thick; hymenium not at all lamellate .....*Trametes p.* 138
8. Plants coriaceous or corky, less than 1 cm. thick; hymenium often lamellate .....*Lenzites p.* 145
9. Hymenium broken up into teeth..... 10
9. Hymenium poroid, not broken up into teeth..... 13
10. Tubes or teeth 5 mm. or more long.....*Irpex p.* 151
10. Tubes or teeth less than 5 mm. long..... 11
11. Hymenium labyrinthiform at first and remaining so at the margin *Dædalea p.* 143
11. Hymenium never labyrinthiform..... 12
12. Pileus less than 1 cm. broad; fungus mostly resupinate.....*Irpex p.* 151
12. Pileus more than 1 cm. broad; fungus not mostly resupinate *Polyporus p.* 86
13. Pores large and hexagonal; stipe present..... 14
13. Pores small and circular or angular; stipe present or absent..... 15
14. Stipe lateral, often rudimentary; pores usually radiating and longer in the radial direction.....*Favolus* p. 148
14. Stipe usually central or subcentral; pores not radiating....*Polyporus p.* 86
15. Tubes in a single layer; plants annual..... 16
15. Tubes in two to several layers; plants perennial 17

[1]See Introduction p. 82.

16. Tubes not in a distinct stratum but appearing to be sunken to different depths into the context ..... *Trametes p.* 138
16. Tubes forming a well marked stratum entirely distinct from the context ..... *Polyporus p.* 86
17. Hymenium bright yellowish brown; plants growing only on the wood of coniferous trees ..... *Trametes p.* 138
17. Hymenium whitish, flesh-colored, dull brown, etc., but not bright yellowish brown; plants growing on the wood of either coniferous or deciduous trees ..... *Fomes p.* 126

DESCRIPTIONS AND KEYS TO THE SPECIES

POLYPORUS Mich. ex Fries,
Syst. Myc. 1: 341. 1821; Mich. Nov. Plant. Gen. 129. 1729.

Plants annual or in rare cases persisting for two or three years, terrestrial or epixylous, sessile or stipitate; pileus fleshy, coriaceous or corky in texture, small or of immense size, often brightly colored; context white, yellow, red, or brown; tubes in a single layer, all sunken into the context to an equal depth so that their bases form a definite continuous straight line; mouths mostly circular or angular, in rare cases showing a favoloid or daedaloid tendency and sometimes breaking up into teeth; stipe (when present) variable in position and texture; spores white (bluish in one species), or some shade of brown.

KEY TO THE SPECIES

Context white or whitish ..... Section I.
Context reddish or yellowish ..... Section II.
Context brown or brownish ..... Section III.

Section I.

Sporophore stipitate or substipitate 1
Sporophore sessile or sometimes effused-reflexed but never stipitate 21
1. Pileus and stipe covered with a reddish varnish 2
1. Pileus and stipe not red-varnished 3
2. Varnish disappearing with age, the pileus then whitish or yellowish *61. P. Curtisii*
2. Varnish persisting, the pileus not changing color ..... *60. P. lucidus*
3. Plant small, not more than 1 cm. high ..... *29. P. pocula*
3. Plant always much larger 4
4. Stipe compound, branching near the base; pileoli usually several or many. 5
4. Stipe simple or not branching more than once; pileus generally single 9
5. Pileoli small (usually less than 5 cm. broad) and numerous 6
5. Pileoli large (5 cm. or more broad) and few in number 7

6. Pileoli regular in outline and centrally attached; the branches of the stipe regular and cylindrical in form ..... *38. P. umbellatus*
6. Pileoli always laterally attached; the stipe branches irregular. *39. P. frondosus*
7. Spores roughly echinulate ..... *41. P. Berkeleyi*
7. Spores smooth 8
8. Pileus pallid or light brown; hymenium usually turning black where bruised and on drying ..... *40. P. giganteus*
8. Pileus yellowish green; hymenium not turning black ..... *37. P. flavovirens*
9. Context soft and spongy above, firm next to the hymenium; plants often much distorted; usually growing about stumps ..... *28. P. distortus*
9. Context uniform; plants not distorted 10
10. Plants growing on the ground 11
10. Plants growing on wood 12
11. Stipe black and rooting at the base; pileus some shade of brown ..*36. P. radicatus*
11. Stipe not black and rooting at the base; pileus yellowish green ..*37. P. flavovirens*
12. Sporophore more or less globose; tubes concealed by a volva ..*27. P. volvatus*
12. Sporophore not globose; volva absent 13
13. Sporophore arising from a cup-shaped, sterile body that sometimes disappears; pileus white; found only on dead branches of *Ulmus* ..... *6. P. conchifer*
13. Sporophore not arising from a cup-shaped sterile body 14
14. Margin of the pileus projecting 5 mm. or more beyond the hymenium; hymenium separating smoothly from the context in fresh specimens; growing only on *Betula* ..... *26. P. betulinus*
14. Plants not as above 15
15. Hymenium bright sulphur-yellow ..... *42. P. sulphureus*
15. Hymenium not bright sulphur-yellow 16
16. Mouths of the tubes minute, averaging 4-7 to a mm. 17
16. Mouths of the tubes larger, averaging 1-3 to a mm. 18
17. Mouths of the tubes averaging 4 to a mm.; pileus rarely more than 5 cm. in diameter ..... *35. P. elegans*
17. Mouths of the tubes averaging about 6 to a mm.; pileus 4–20 cm. in diameter *34. P. picipes*
18. Pileus large, more than 5 mm. thick; plant growing on living trees; stipe black at the base ..... *33. P. squamosus*
18. Pileus small or medium sized, not more than 5 mm. thick; stipe not black at the base 19
19. Tubes long-decurrent on the stipe; context soft and friable when dry *32. P. pennsylvanicus*
19. Tubes slightly or not at all decurrent; context not soft and friable when dry .. 20
20. Pileus yellowish brown; mouths of the tubes almost 1 mm. in diameter; walls thin ..... *31. P. arcularius*
20. Pileus darker than above, sometimes sooty-black; mouths of the tubes averaging 2 to a mm.; walls at first thick ..... *30. P. brumalis*
21. Pileus red-varnished, at least when young 22
21. Pileus never red-varnished 23
22. Varnish disappearing with age, the pileus then whitish or yellowish ..... *61. P. Curtisii*
22. Varnish persistent, the pileus not changing color ..... *60. P. lucidus*
23. Sporophore more or less globose; tubes concealed by a volva ..... *27. P. volvatus*
23. Sporophore not globose; volva absent 24

24. Sporophore arising from the under side of a cup-shaped, sterile body; found only on dead branches of *Ulmus* ..... *6. P. conchifer*
24. Sporophore not arising from a cup-shaped, sterile body 25
25. Margin of the pileus projecting 5 mm. or more beyond the hymenium; hymenium separating smoothly from the context in fresh specimens; found only on *Betula* ..... *26. P. betulinus*
25. Plants not as above 26
26. Hymenium bright sulphur-yellow ..... *42. P. sulphureus*
26. Hymenium not bright sulphur-yellow 27
27. Pileus distinctly brown in color; context usually light brown; hymenium changing color when bruised ..... *46. P. resinosus*
27. Pileus not brown in color; hymenium never changing color when bruised .. 28
28. Hymenium more or less smoke-colored or black 29
28. Hymenium not at all smoke-colored or black 32
29. Pileus more than 4 mm. thick 30
29. Pileus not more than 4 mm. thick 31
30. Context fragrant, with the odor of anise ..... *23. P. fragrans*
30. Context not fragrant, odor sometimes disagreeable ..... *24. P. fumosus*
31. Mouths of the tubes angular, minute, averaging 5–7 to a mm.; dissepiments thin ..... *22. P. adustus*
31. Mouths of the tubes circular or subcircular, medium sized, averaging 3–5 to a mm.; dissepiments thick ..... *24. P. fumosus*
32. Context fibrous or coriaceous in fresh plants; pileus never more than 1.5 cm. thick, and usually much thinner 33
32. Context either soft, spongy and full of water or firm and corky, often fragile when dry; pileus often more than 1.5 cm. thick 43
33. Hymenium broken up into teeth 34
33. Hymenium entire or lacerate but not broken up into teeth 36
34. Context more than 1 mm. thick ..... *9. P. biformis*
34. Context not more than 1 mm. thick 35
35. Plants growing only on the wood of coniferous trees ..... *2. P. abietinus*
35. Plants growing only on the wood of deciduous trees ..... *3. P. pargamenus*
36. Context 1 mm. or less thick 37
36. Context more than 1 mm. thick 40
37. Mouths of the tubes minute, averaging 4–6 to a mm.; hymenium never violet or purple 38
37. Mouths of the tubes larger, averaging 2–3 to a mm.; hymenium often violet or purple 39
38. Surface of the pileus villous or velvety; pileus multizonate, generally more than 2 cm. broad ..... *1. P. versicolor*
38. Surface of the pileus densely hirsute; pileus azonate or with one or two zones, generally less then 2 cm. broad ..... *4. P. hirsutulus*
39. Plants growing only on the wood of coniferous trees ..... *2. P. abietinus*
39. Plants growing only on the wood of deciduous trees ..... *3. P. pargamenus*
40. Mouths of the tubes large, averaging 1–2 to a mm. ..... *9. P. biformis*
40. Mouths of the tubes medium sized, averaging 3–4 to a mm. 41
41. Tubes more than 2 mm. long ..... *7. P. pubescens*
41. Tubes not more than 2 mm. long 42
42. Surface of the pileus velvety to hirsute ..... *5. P. hirsutus*
42. Surface of the pileus minutely pubescent or glabrous ..... *8. P. Lloydii*

43. Plants mostly resupinate 44
43. Plants not mostly resupinate 45
    44. Pileus azonate, margin often inrolled . . . . . *10. P. semipileatus*
    44. Pileus zonate, margin always straight . . . . . *21. P zonalis*
45. Pileus corky in texture when fresh, usually rather thick and firm 46
45. Pileus soft and spongy in texture when fresh 49
    46. Pileus distinctly encrusted; hymenium and context pinkish or rosy when fresh; plants usually growing on *Fraxinus* . . . . . *P. fraxineus*[1]
    46. Pileus not encrusted; hymenium and context whitish when fresh; plants not usually on *Fraxinus* 47
47. Pileus more than 2 cm. thick; tubes more than 4 mm. long . . . . *25. P. robiniophila*
47. Pileus not more than 2 cm. thick; tubes not more than 4 mm. long 48
    48. Plants with a sweet anise odor . . . . . *23. P. fragrans*
    48. Plants with no odor, or odor disagreeable . . . . . *24. P. fumosus*
49. Mouths of the tubes minute, averaging 6–7 to a mm.; plants with a sweet acid odor . . . . . *14. P. galactinus*
49. Mouths of the tubes larger, averaging 1–4 to a mm. 50
    50. Pileus generally less than 4 cm. broad 51
    50. Pileus generally more than 4 cm. broad 53
51. Pileus pubescent; mouths of the tubes dentate, lacerate, or irregular 52
51. Pileus glabrous; mouths of the tubes entire; plants with a sweet acid odor *12. P. chioneus*
    52. Pileus and spores (in mass) often bluish or slate-colored; tubes equalling in length the thickness of the context . . . . . *11. P. caesius*
    52. Pileus and spores pure white; tubes shorter in length than the thickness of the context . . . . . *13. P. lacteus*
53. Plants growing only on the wood of coniferous trees 54
53. Plants growing only on the wood of deciduous trees 55
    54. Tubes usually more than 5 mm. long, the mouths averaging 2–3 to a mm. *19. P. borealis*
    54. Tubes usually less than 5 mm. long, the mouths averaging 4–5 to mm. *18. P. guttulatus*
55. Margin of the pileus thick and rounded . . . . . *17. P. obtusus*
55. Margin of the pileus thin and acute 56
    56. Mouths of the tubes large, averaging 1–2 to a mm. . . . . *16. P. delectans*
    56. Mouths of the tubes small, averaging 3–5 to a mm. 57
57. Fresh plant with a disagreeable odor; context very hard when dry . *20. P. Spraguei*
57. Fresh plant with no disagreeable odor . . . . . *15. P. spumeus*

Section II

Pileus and hymenium deep cinnabar-red 1
Pileus and hymenium not deep cinnabar-red (rosy or orange-colored in some species) 2
1. Pileus less than 5 mm. thick, often zonate . . . . . *44. P. sanguineus*
1. Pileus more than 5 mm. thick, never zonate . . . . . *45. P. cinnabarinus*
    2. Hymenium bright sulphur-yellow in fresh plants . . . . . *42. P. sulphureus*
    2. Hymenium not bright sulphur-yellow 3
3. Plant growing only on the wood of *Quercus* and *Castanea*; pileus yellowish or orange-colored . . . . . *43. P. Pilotæ*

[1] For description see p. 130 under the genus *Fomes*.

3. Plant growing usually on *Fraxinus;* pileus usually stained more or less with red ..... *P. fraxineus*[1]
3. Plant growing usually on coniferous wood; rose-colored without and within ... *P. carneus*[2]

Section III

Pileus stipitate or substipitate 1
Pileus sessile or effused-reflexed, not stipitate 9
1. Pileus and stipe covered with a reddish varnish at least when young 2
1. Pileus and stipe not red-varnished 3
2. Pileus and stipe at first red-varnished, the varnish disappearing and the pileus becoming whitish or yellowish when mature ..... *61. P. Curtisii*
2. Pileus and stipe strongly red-varnished, the varnish not disappearing with age ..... *60. P. lucidus*
3. Context not more than 1 mm. thick; plants growing on the ground 4
3. Context more than 1 mm. thick; plants growing on wood or attached to buried wood 6
4. Surface of the pileus marked with silky striations ..... *59. P. cinnamomeus*
4. Surface of the pileus not silky 5
5. Mouths of the tubes small, averaging 2–4 to a mm.; tubes usually less than 3 mm. long ..... *58. P. perennis*
5. Mouths of the tubes large, averaging 0.5–1 mm. or more in diameter; tubes usually more than 3 mm. long ..... *57. P. focicola*
6. Surface of the pileus distinctly encrusted ..... *P. lobatus*[3]
6. Surface of the pileus not at all encrusted 7
7. Context decidedly duplex, spongy above, firm next to the tubes ... *55. P. circinatus*
7. Context not duplex 8
8. Hymenium some shade of yellow (yellowish brown, yellowish green, etc.), quickly changing color when bruised; growing on or about trees and stumps of *Pinus*; spores white ..... *54. P. Schweinitzii*
8. Hymenium cinereous to brownish, not changing color when bruised; growing on the ground or attached to buried wood; spores brown ..... *56. P. obesus*
9. Pilei forming a densely imbricate, globose or cylindrical mass ..... *P. graveolens*[4]
9. Pilei not forming a densely imbricate, globose or cylindrical mass 10
10. Pileus red-varnished ..... *60. P. lucidus*
10. Pileus not red-varnished 11
11. Pileus distinctly encrusted ..... *P. lobatus*[5]
11. Pileus not distinctly encrusted 12
12. Plants growing on or about stumps or trunks of *Pinus* ... *54. P. Schweinitzii*
12. Plants growing on wood of deciduous shrubs or trees, often on living trunks 13
13. Context usually less than 7 mm. thick; plants small or medium sized 14
13. Context more than 7 mm. thick; plants large 17
14. Spores white 15
14. Spores brown 16

[1]See p. 130 for a description of this plant.
[2]For description of this plant see p. 131 under the genus *Fomes*.
[3]This plant is described on p. 137 under the genus *Fomes*.
[4]For description see p. 131 under the genus *Fomes*.
[5]For description see p. 137 under the genus *Fomes*.

15. Pileus spongy and watery when fresh; context friable when dry; mouths of the tubes averaging 2–4 to a mm. ..... *47. P. nidulans*
15. Pileus firm and rigid; context corky when dry; mouths of the tubes minute, averaging 5–8 to a mm. ..... *48. P. gilvus*
16. Plants growing on the wood of *Alnus* and *Betula*; spores light brown... *49. P. radiatus*
16. Plants growing on the wood of *Acer*, *Fagus*, and other deciduous trees... *50. P. cuticularis*
17. Context very light brown ..... *46. P. resinosus*
17. Context yellowish brown or darker 18
18. Surface of the pileus hirsute; plants growing on various diseased deciduous trees ..... *51. P. hispidus*
18. Surface of the pileus fibrillose or glabrous; plants growing only on the wood of *Quercus* 19
19. Sporophore medium sized, less than 10 cm. broad and 3 cm. thick..... *53. P. dryophilus*
19. Sporophore large, more than 10 cm. broad and 3 cm. thick..... *52. P. dryadeus*

1. P. versicolor L. ex Fries, Syst. Myc. 1: 368. 1821.

Boletus versicolor L. Sp. Plant. 1176. 1753.

Pileus sessile or effused-reflexed, imbricate or single, dimidiate or encircling twigs and then often orbicular by confluence, 2–5 x 2–7 x 0.1–0.3 cm., coriaceous, prevailing color grayish, but marked by many narrow, multicolored zones, ranging from white to yellow, brown, reddish, greenish, blackish, etc., villous or velvety, the margin thin and acute, usually sterile below; context white or whitish, fibrous, less than 1 mm. thick; tubes 1–2 mm. long, the mouths white or yellowish, sometimes somewhat glistening, circular to angular, averaging 3–5 to a mm., the walls thin, entire or slightly lacerate; spores white, smooth, oblong, sometimes curved, 1.2–2 x 5–6.3 μ.

On all kinds of dead wood. Common throughout the year.

Easily distinguished by the multizonate, multicolored pileus. *P. hirsutulus* Schw. is often considered to be a form of this species. *P. zonatus* Fries, as reported by Morgan, is one of the many forms of it. The following references contain good illustrations of our plant: Hard, Mushrooms *f. 343.*, White, Hymen. Conn. *pl. 36.*, and Moffatt, Higher fungi of the Chicago region *pl. 17. f. 1.*

2. P. abietinus Dicks. ex Fries, Syst. Myc. 1: 370. 1821.

Boletus abietinus Dicks. Fasc. Pl. Crypt. Brit. 3: 21. 1793.

Pileus sessile or effused-reflexed, dimidiate and broadly attached, or flabelliform and attached by the attenuate base

of the pileus, 0.5–5 x 0.5–5 x 0.1–0.2 cm., coriaceous, white to cinereous or almost black behind, villous, zonate, margin thin and acute; context white or pallid, fibrous, not more than 1 mm. thick; tubes less than 3 mm. long, the mouths white to bay and often violaceous toward the margin, averaging 2–3 to a mm., the dissepiments thin and soon lacerate and breaking up into teeth.

Growing only on the wood of coniferous trees. In autumn. Rare.

Closely related to *P. pargamenus* Fries, from which it is most easily separated by the habitat. The following spore dimensions are found in the literature: Karsten—"oblong 4–6 x 1–3 μ"; Murrill—"globose, smooth, hyaline, 4.5–5.5 μ in diameter"; Bresadola—"hyaline, cylindrical, subcurved, 6–7 x 2.5 μ."

3. P. pargamenus Fries, Epicr. Syst. Myc. 480. 1838.

Pileus sessile or effused-reflexed, imbricate, dimidiate or flabelliform, sometimes attached by an attenuate base, 1–7 x 1–7 x 0.1–0.4 cm., coriaceous, whitish to cinereous or yellowish brown, villous, zonate, the zones sometimes differently colored, margin very thin, acute, broadly sterile below, often violaceous in color; context white or whitish, fibrous, very thin, less than 1 mm. thick; tubes not more than 2.5 mm. long, the mouths whitish to bay and often violaceous toward the margin, angular, averaging 2–3 to a mm., the dissepiments thin and soon breaking up into teeth; spores white, smooth, oblong, slightly curved, 2–2.5 x 5–6.3 μ.

Growing on the wood of deciduous trees, especially of *Quercus* and *Prunus*. September to December. Common.

Close to *P. abietinus* Dicks. ex Fries, but usually found on dead wood of deciduous trees. Well represented by Hard (Mushrooms *f. 345*) as *P. pergamenus*.

4. P. hirsutulus Schw. Trans. Am. Phil. Soc. II. 4: 156. 1832.

Pileus sessile or effused-reflexed, often imbricate, dimidiate, 0.5–2 x 0.5–2.7 x 0.1–0.2 cm., coriaceous, gray or cinereous to yellowish brown, hirsute or strigose, azonate or with 2–3 colored zones, margin thin and acute, usually sterile below; context white or whitish, membranous, less than 1 mm. thick; tubes less than 2 mm. long, mouths whitish to yellowish, rarely

glistening, circular or angular, averaging 3–5 to a mm., the dissepiments thin and entire.

On dead branches of deciduous trees, more often on fruit trees. Found from August to December. Not common.

Separated from *P. versicolor* L. ex Fries, by the more hirsute or strigose pubescence on the pileus, and by the smaller size. Specimens collected at Cincinnati by D. L. James and referred to *P. velutinus* Fries are now referred to this species.

5 P. hirsutus Wulfen, ex Fries, Syst. Myc. **1**: 367. 1821.

Boletus hirsutus Wulfen, in Jacq. Coll. **2**: 149. 1788.

Pileus sessile, or effused-reflexed, dimidiate, 1.5–5 x 1.5–7 x 0.2–1 cm., flexible when moist, firm and sometimes rigid when dry, grayish to yellowish or smoky brown, hirsute or tomentose, sometimes zonate, sometimes concentrically sulcate, the margin thin or rather thick, acute, sometimes dark colored; context white or pallid, tough to soft-corky, 1–6 mm. thick; tubes 1–4 mm. long, the mouths white, grayish or fuliginous, circular to somewhat angular, averaging 3–4 to a mm., the walls rather thick and always entire; spores white, smooth, cylindrical, often curved, 2.5 x 5–8 μ.

On dead wood of deciduous trees. Found throughout the year.

From closely related species with a conspicuous hairy covering this plant is perhaps most easily separated by the persistently thick walled tubes that never become torn or lacerate. Any plant with the characteristics of this group and possessing the dark-colored marginal band to which reference is made in the description may always with safety be referred to this species. From *P. versicolor* L. ex Fries, the plant is separated by the absence of the numerous multicolored zones. Hard's figure (Mushrooms *f. 342*) is not a good illustration of our plant. Murrill describes the plant under the name of *Coriolus nigro-marginatus* (Schw.) Murr.

6. P. conchifer Schw. ex Fries, Epicr. Syst. Myc. 463. 1838.

Boletus conchifer Schw. Syn. Fung. Car. 98. 1822.

Pileus sessile or attached by a lateral tubercle and then appearing substipitate, reniform to dimidiate in outline, 1–3 x 1–4 x 0.1–0.3 cm., coriaceous, white to yellowish, glabrous,

zonate or azonate, the margin very thin and acute; on the upper surface and at the base of the pileus a small cup-shaped or disk-like sterile structure is usually borne, white or brown and often zoned on the inside; context white, fibrous, less than 1 mm. thick; tubes not more than 2 mm. long, at first white, often yellowish on drying, the mouths angular and thin-walled averaging about 3 to a mm., the dissepiments often lacerate; stipe (?) rudimentary, tubercular; spores not obtained.

Growing only on fallen branches of *Ulmus*. Common.

This plant has somewhat the appearance of *P. pubescens* Schum. ex Fries, from which, however, it is easily separated by the much thinner pileus, the attenuate base, the presence of the sterile cup, and the habitat. The cup is sometimes absent. The development of the cup has not been closely followed. Lloyd believes that the fertile pileus is first developed and from it the sterile cup arises, and that during the winter the fertile portion falls away, the cup persisting on the substratum but not giving rise to new pilei the next season. Miss Ames comes to the conclusion that the sterile cups represent pilei whose marginal hyphæ have been killed by unfavorable conditions and which as a result may develop a fruiting surface from the base of the dead cup-like pileus. This would explain the occasional absence of the sterile cup, its presence depending upon the death of the marginal hyphæ in the early stages of the production of a first pileus. *P. virgineus* Schw. described from North Carolina is said to be this plant. The plant is exceptionally well illustrated by Lloyd (Myc. Notes, Polyporoid Issue 3 *f. 365–66*), and by Moffat (Higher fungi of the Chicago region *pl. 16. f. 2*).

7. P. pubescens Schum. ex Fries, Syst. Myc. **1**: 367. 1821.

Boletus pubescens Schum. Enum. Pl. Saell. **2**: 384. 1803.

Polyporus Sullivantii Mont. Ann. Sci. Nat. II. **18**: 243. 1842.

Pileus sessile, dimidiate, 1.5–5 x 2.5–5 x 0.4–1 cm., fleshy-tough when fresh, firm when dry, white or yellowish in fresh specimens, sometimes umber or brown when dry, villous-tomentose, zonate or azonate, margin thin, acute; context white or pallid, fibrous-tough when fresh, more firm when dry, 1–5 mm. thick; tubes 1–4 mm. long, the mouths white, yellowish, or umber, angular, averaging 3–4 to a mm., the

dissepiments thin, entire to dentate; spores white, smooth, cylindrical, curved, 2.7–3.6 x 5.4 μ.

On dead wood of deciduous trees. August to November. Common.

Plants collected in the Miami valley by Morgan and referred by him to *P. velutinus* Fries belong here. Plants distributed by Kellerman in his 'Fascicles of Ohio Fungi' as *P. molliusculus* Berk. are referred to this species. *P. fibula* Fries as reported by Morgan is probably the same as *P. pubescens* var. *Grayii*, here included under *P. pubescens*. Hard (Mushrooms *f. 339*) gives a good illustration of the plant.

8. P. Lloydii (Murr.) Overholts, n. comb.

Coriolus Lloydii Murrill, N. Am. Flora **9**: 23. 1907.

Pileus rather thin, laterally connate, rigid, tough, cuneate to flabelliform, applanate, tubercular-sessile, 2–3 x 3–4 x 0.2–0.4 cm.; surface white or isabelline, scabrous, somewhat rugose, marked with a few narrow, indistinct, pale latericeous zones; margin thin, fertile, irregular, lobed; context punky-fibrous, white, 1.5–2 mm. thick; tubes 1–1.5 mm. long, white within, mouths angular, subglistening, 4 to a mm., edges thin, firm, dentate, white or isabelline; spores globose, smooth, hyaline, 2 μ; hyphæ 5 μ.

On dead wood. Rare.

The above description is taken from the 'North American Flora.' The type specimens were collected near Cincinnati, Ohio, by C. G. Lloyd, and to the writer's knowledge the plant has not been found since. The species appears to be distinct.

9. P. biformis Klotzsch, Linnaea **8**: 486. 1833.

P. molliusculus Berk. Hooker's Lond. Jour. Bot. **6**: 320. 1847.

Plants sessile, effused-reflexed or resupinate, often imbricate; pileus dimidiate or laterally confluent and elongate, 0–5.5 x 1.5–6 x 0.2–1.5 cm., soft and pliable when fresh, slightly flexible to rigid when dry, white, pallid, bay, or ochraceous, appressed-fibrillose, usually rough, azonate or subzonate, the margin thin and acute; context white or whitish, fibrous-tough when fresh, soft-corky when dry, 1–5 mm. thick; tubes white, becoming bay on drying, 2–5 mm. long, the mouths circular to angular or sinuous, averaging 1–2 to a mm., the dissepiments

rather thin and usually becoming lacerate and broken up into teeth at an early stage of growth, sometimes remaining poroid, especially toward the margin of the pileus; spores white, smooth, oblong, curved, 2–2.6 x 7–8 μ.

Growing on old logs. September to December. Common.

The following group of characters will usually identify the species: the semi–resupinate habit of growth, whitish or tan-colored pileus, and the rather long tubes with large mouths, soon breaking up into teeth. *P. molliusculus* was named by Berkeley from specimens sent to him from Ohio by Lea. Morgan's determination of *P. molliusculus* was an error, his plants belonging to *P. pubescens* Schum. ex. Fries. Kellerman repeated the error in distributing *P. molliusculus* in his 'Ohio Fungi Fascicles.' For illustration see Hard, Mushrooms *f. 341*.

10. P. semipileatus Peck, Ann. Rept. N. Y. State Mus. **34**: 43. 1881.

Plants resupinate or effused-reflexed, rarely strictly sessile; pileus dimidiate or elongate, 0–1.5 x 0.7–3.5 x 0.1–0.5 cm., soft and spongy when fresh, rigid when dry, white, yellowish, or reddish brown, slightly tomentose to glabrous, azonate, margin thin, acute; context whitish, soft when fresh, firm when dry, 1–4 mm. thick; tubes less than 2 mm. long, the mouths white, greenish or somewhat violaceous, angular, minute, averaging 4–6 to a mm., the walls entire; spores white, smooth, oblong, curved, 1 x 3–4 μ.

On old limbs on the ground. September to December. Rare.

Easily recognized by the minute pores, the semi–resupinate habit of growth, and the often violet tinted hymenium. There is no previous record of the plant occurring in Ohio. Collections were made at Oxford, in 1911, for the first time.

11. P. caesius Schrad. ex Fries, Syst. Myc. **1**: 360. 1821.

Boletus caesius Schrad. Spic. Fl. Ger. 167. 1794.

Pileus sessile or effused-reflexed, dimidiate, 1–3.5 x 2–6 x 0.3–2 cm., soft and spongy when fresh, rigid when dry, whitish to cinereous, often with a bluish tinge, distinctly villous or tomentose especially behind; azonate, margin thin and acute; context white, soft, spongy and full of water when fresh, friable when dry, 0.3–1 cm. thick; tubes 2–7 mm. long, mouths white, pallid, or bluish gray, angular, averaging 3–5 to a mm., the

walls thin and usually lacerate; spores minute, white, smooth, cylindrical, sometimes curved, 1.2–1.5 x 4.7–5.2 μ.

On dead wood of deciduous and coniferous trees. October to December. Rare.

The bluish color of the pileus and hymenium is so often wanting that other characters must frequently be used in the identification of the plant. The slender tubes, usually longer than or as long as the thickness of the context, is apparently a rather constant character of the plant. The villous or tomentose pileus separates it from *P. chioneus* Fries and *P. lacteus* Fries and these are the only species with which it is likely to be confused.

12. P. chioneus Fries, Syst. Myc. 1: 359. 1821.

Pileus sessile, dimidiate, 1–3 x 2–5 x 0.5–3 cm., soft and spongy when fresh, rigid when dry, whitish to grayish or yellowish, azonate, glabrous or with a slight strigose tomentum towards the base, sometimes covered with a thin grayish or yellowish pellicle that becomes more evident on drying; margin acute, sometimes inflexed on drying; context white, soft and spongy when fresh, fragile when dry, 0.3–2 cm. thick, azonate, with a sweet acid odor; tubes 1–8 mm. long, mouths white or yellowish, usually glistening, angular, averaging 3–4 to a mm., the walls thin but entire; spores white, smooth, oblong, slightly curved, 1–1.7 x 4–5 μ.

On dead wood. September to November.

From *P. galactinus* Berk. this plant is most easily separated by the oblong, curved spores. The usually glabrous pileus and the absence of bluish tints separates it from *P. caesius* Schrad. ex Fries. Whether it is distinct from *P. lacteus* Fries may well be doubted. The plant is much in dispute in Europe. Our plants have been described as *P. albellus* Peck.

13. P. lacteus Fries, Syst. Myc. 1: 359. 1821.

Pileus pure white, fleshy-fibrous, fragile, triangular, pubescent, azonate externally and internally, margin inflexed, acute; pores thin acute, dentate, becoming torn and labyrinthiform. Commonly small and thin but sometimes large and transversely elongate, often gibbous behind, becoming glabrate and uneven. (Adapted from Fries, Hymen. Eur. 546.)

On dead wood of deciduous trees. Rare.

Until quite recently this and the preceding species have been held to be quite distinct. Of late years the European mycologists are coming to believe that they cannot be regarded as distinct species. Murrill would separate them on the ground that *P. chioneus* always has a distinct cuticle which is entirely lacking in *P. lacteus*. The writer has endeavored to keep the plants distinct on the basis of the differences noted by Fries. If this proves unfeasible then the two must be united as one species under the name of *P. chioneus*, at least with reference to their occurrence in this country.

14. P. galactinus Berk., Hooker's Lond. Jour. Bot. **6**: 321. 1847.

Pileus sessile, imbricate or single, dimidiate, 3–7 x 3–7 x 0.5–2 cm., soft and pliant when fresh, more or less watery, rigid and contorted on drying, white, grayish, or somewhat yellowish, tomentose to strigose-tomentose, especially at the base, becoming glabrous with age, azonate, margin thin and acute; context white or pallid, watery and spongy when fresh, with a distinct sweet acid odor, firm when dry, sometimes more or less duplex, 3–8 mm. thick; tubes 2–7 mm. long, mouths white to bay, often glistening, circular to angular or sinuous, minute, averaging about 6 to a mm.; spores white, smooth, ellipsoid, 2–2.5 x 3.5–4 μ, uninucleate and with a very transparent wall.

Growing on dead wood of deciduous trees. August to November. Common.

The sweet acid odor mentioned in the description is a distinguishing character of all collections of this species. No mention is made of the odor in any published work to the writer's knowledge, except in Peck's description of *P. immitus* in which the odor is described as subacid. *P. immitus* is in all probability this plant. The odor is so constant that whenever it is noticed in connection with any minute-pored form of this section one can be sure that the plant belongs to this species.

All of the collections that I have referred to this species are watery when fresh, have a sweet acid odor, and when dried shrink much in size and often become much contorted. The context becomes thin and hard and takes on a resinous, dark brown or black color. This appearance may be uniform through

the context or the dark resinous color may be limited to a narrow line next to the hymenium or confined to two or three narrow zones in the context. It is difficult to distinguish these species with a white watery context and the writer's presentation of them may be open to criticism.

15. P. spumeus Sow. ex Fries, Syst. Myc. 1:358. 1821.

Boletus spumeus Sow. Col. Fig. Eng. Fungi *pl. 211*. 1797.

Pileus sessile, dimidiate, watery and fleshy-tough when fresh, firm when dry, 7–12 x 10–20 x 2–3 cm., much smaller on drying, appearing appressed-tomentose, white or grayish, somewhat yellowish or brownish on drying, azonate, margin rather thick but acute; context white, soft, spongy, and full of water, rather fragile on drying, more or less zonate, 1–3 cm. thick; tubes 0.5–1.5 cm. long, mouths white or yellowish on drying, angular, averaging 3 to a mm.; spores white, smooth, globose, or subglobose, 4.5–5.2 μ in diameter, distinctly uninucleate.

Growing on injured or diseased deciduous trees, especially *Ulmus* and *Acer*. October and November. Rare.

The plant is closely related to *P. delectans* Peck, with the same habitat and general appearance, but separated from that species by the smaller mouths of the tubes and by the distinctly uninucleate and more globose spores. The plants so referred do not agree with the figure given by Sowerby, nor with Fries' description. My plants were determined by Bresadola.

16. P. delectans Peck, Bull. Torr. Bot. Club 11:26. 1884.

Pileus sessile, sometimes imbricate, dimidiate in outline, 3–7 x 4–15 x 0.7–3 cm., rather spongy and watery when fresh, firm and rigid when dry, white or whitish, finely tomentose or glabrous, azonate, margin thin and acute; context white, in large specimens duplex, with a firm lower layer and a soft upper layer, in smaller specimens more uniform, 0.5–1.5 cm. thick; tubes 0.5–1.5 cm. long, mouths white, yellowish on drying, circular to angular, large, averaging 1–2 to a mm.; spores white, smooth, ellipsoid to globose, 4.5–5.5 x 6.5–8.5 μ.

On diseased or injured trunks of deciduous trees, especially *Acer;* sometimes on logs of *Fagus*. September to December. Frequent.

The species is separated from *P. spumeus* Sow. ex Fries by the larger tube mouths and the less globose spores that have

not been observed to be uninucleate as in that species. It is a large white fungus distinct from the other allied species in size, length of tubes, and habitat.

17. P. obtusus Berk. Ann. & Mag. Nat. Hist. I. **3**: 390. 1839.

Plants annual, sessile, sometimes imbricate; pileus dimidiate, convex or ungulate, 3–9 x 4–15 x 3–6 cm., somewhat spongy when fresh, firm, rigid, and very light in weight when dry, cinereous to yellowish or darker in herbarium specimens, hirtose-tomentose, rarely becoming glabrous, azonate, margin thick, obtuse; context white or whitish, spongy to corky, sometimes duplex, 1–3 cm. thick; tubes 1.5–3 cm. long, the mouths white, bay or brown on drying, circular to angular and sinuous, 1 mm. or more in diameter; spores (teste Murrill) globose, smooth, hyaline, 6–8 μ.

On trunks of diseased deciduous trees, especially *Quercus*. Rare.

Always easily recognized by the rounded and obtuse margin, and the long tubes with large mouths. Excellent illustrations are given by Spaulding (Ann. Rept. Mo. Bot. Gard. **16**: *pl. 13–19*).

18. P. guttulatus Peck, in Sacc. Syll. Fung. **6**: 106. 1888.

P. maculatus Peck, Ann. Rept. N. Y. State Mus. **26**: 69. 1874.

Pileus sessile, sometimes imbricate, dimidiate, 3–8 x 5–12 x 0.4–1.5 cm., soft and fleshy when fresh, firm and rigid when dry, white to yellowish or slightly brownish, glabrous, azonate or sometimes zonate on the margin, sometimes marked with rounded depressed spots, margin thin, acute; context white or pallid, soft and fleshy when fresh, soft-corky or friable when dry, 0.4–1 cm. thick; tubes 1–5 mm. long, the mouths white to yellowish or umbrinous, angular, averaging 4–5 to a mm.; spores white, smooth, oblong-ellipsoid, 2.5–3 x 3–5 μ. (Cf. Murrill, globose, smooth, hyaline, 5 μ in diameter.)

Growing on wood of coniferous trees. Rare.

The distinguishing character of the species is the presence of the round depressed spots on the pileus.

19. P. borealis Fries, Syst. Myc. **1**: 366. 1821.

Pileus sessile, dimidiate, sometimes with an attenuate base, 3–8 x 4–12 x 0.5–2.5 cm., somewhat watery and spongy when fresh, rigid when dry, white or yellowish, sometimes brownish, hispid to tomentose, azonate, margin thin and acute; context

white or yellowish, distinctly duplex, firm and fibrous below, soft and floccose above, 0.5–2 cm. thick; tubes 3–10 mm. long, the mouths white or yellowish, angular to irregular and uneven, rather large, averaging 2–3 to a mm.; spores (teste Murrill) ovoid, smooth, hyaline, 5–6 x 3–4 μ.

Growing only on trunks of coniferous trees. Rare.

The species is most easily separated from its allies by the size and habitat. For illustrations see Atkinson, Mushrooms *f. 9.*, Duggar, Fung. Dis. Plants *f. 228.*, and Atkinson, Cornell Univ. Agr. Exp. Sta. Bul. 193: *f. 63.*

20. P. Spraguei Berk. & Curt. Grevillea **1**: 50. 1872.

Plants annual, sessile or decurrent, sometimes imbricate; pileus dimidiate, 4–12 x 4–10 x 0.6–2 cm., fleshy-tough when fresh, rigid when dry, white or cinereous, appressed-tomentose or glabrous, azonate or somewhat zonate, margin thin or rather thick, acute, often blackening on drying; context white, watery, tough-fibrous when fresh, sometimes very hard when dry, zonate, 0.3–1.5 cm. thick, with a disagreeable odor in fresh specimens; tubes 0.3–1 cm. long, mouths white or discolored, circular or angular, averaging 3–4 to a mm.; spores (teste Murrill) ellipsoidal smooth, hyaline, 6 x 4 μ.

On dead wood of deciduous trees, especially on *Fagus*, *Quercus*, and *Castanea*. July to September. Common.

Fresh specimens are always easily distinguished by the very disagreeable odor. Dried plants are characteristically very hard and rigid, the context almost bony in texture.

21. P. zonalis Berk. Ann. & Mag. Nat. Hist. I. **10**: 375. 1842.

Plants annual, sessile, effused-reflexed, or entirely resupinate; pileus dimidiate or laterally confluent, 0–2.5 x 1–5 x 0.2–0.5 cm., fleshy and pliable when fresh, rigid and firm when dry, whitish to flesh-colored or isabelline, finely tomentose to glabrous, at first azonate but becoming zoned when mature, the margin at first thick, thin with age; context white, fibrous when fresh, hard and rigid when dry, 1–2 mm. thick; tubes 1–3 mm. long, the mouths usually more or less flesh-tinted when fresh, angular, averaging 4–5 to a mm., the walls thick and entire, very firm and rigid on drying; spores white, smooth, globose, 2.5–5 μ broad, with one large nucleus.

On old rotting logs, especially of *Liriodendron*. August to December. Not common.

The writer has collected this plant several times in the Miami valley, almost always on logs of *Liriodendron tulipifera*. The plant is usually entirely resupinate and has doubtless been described as a *Poria*, but good collections were made which showed beyond a doubt the pileate tendency of the plant. No disposition could be made of the plant until Dr. Murrill suggested that it might belong to *P. zonalis*. Later, specimens were sent to Rev. Bresadola who pronounced it that species and an opinion recently received from Mr. Lloyd expresses the same view. It is, however, quite different from the usual forms of that plant and the name is used with some apprehension. The plant is also abundant in Missouri where the writer has found the pileate forms to be much more common than in Ohio. *P. zonalis* has been supposed to be confined to the Gulf States in this country, although it is not surprising that semi-tropical forms found there should extend their range up the large river valleys to the north.

22. P. adustus Willd. ex Fries, Syst. Myc. 1: 363. 1821.

Boletus adustus Willd. Fl. Berol. 392. 1787.

Plants annual, sessile, effused-reflexed, or resupinate; pileus dimidiate, often imbricate, 1–6 x 2–7 x 0.2–0.4 cm., fleshy-tough when fresh, coriaceous or rigid when dry, white to cinereous or pale tan, fibrillose-tomentose to almost glabrous, zonate or azonate, the surface usually rough, margin thick and broadly sterile below when young, becoming thin when mature; context white or pallid, rather soft when fresh, corky or fibrous-corky when dry, 1–3.5 mm. thick; tubes not more than 1 mm. long, the mouths grayish black to black, angular, even, minute, averaging 5–7 to a mm.; spores white, smooth, oblong to oblong-ellipsoid, 2–2.5 x 3.8–4.3 μ.

On stumps and trunks of dead deciduous trees. August to December.

This species differs from *P. fumosus* Pers. ex Fries and *P. fragrans* Peck in the smaller size and the uniformly black hymenium.

23. P. fragrans Peck, Rept. N. Y. State Museum **30**: 45. 1879.

Plants annual, sessile or effused-reflexed; pileus dimidiate, imbricate, 2–8 x 4–10 x 0.5–2 cm., fleshy-tough when fresh, firm and rigid when dry, cinereous to reddish gray, finely tomentose to almost glabrous, subzonate or azonate, the margin thin and acute; context whitish or pallid, tough when fresh, soft-corky when dry, 4–8 mm. thick, with a sweet anise-like odor that persists in dried plants; hymenium sometimes separated from the context by a narrow, dark-colored line; tubes less than 4 mm. long, the mouths whitish or somewhat smoke-colored, blackish when bruised, angular, the dissepiments becoming dentate and the mouths unequal in size, averaging 3–4 to a mm.; spores (teste Murrill) white, globose to ovoid, smooth, 5–6 μ in diameter.

On stumps and trunks, especially of *Ulmus*. Frequent.

The distinguishing characters of this species are the fragrant odor and the unequal and irregular pores—characters which separate it from *P. adustus* and *P. fumosus*. The name *P. puberula* Berk. & Curtis is sometimes applied to this plant.

24. P. fumosus Pers. ex Fries, Syst. Myc. **1**: 367. 1821.
Boletus fumosus Pers. Syn. Fung. 530. 1801.

Plants annual, sessile or effused-reflexed; pileus dimidiate, often imbricate, 2–7 x 3–8.5 x 0.3–2 cm., somewhat fleshy-tough when fresh, firm and rigid when dry, grayish to very pale tan-colored, finely tomentose, subzonate or azonate, margin thin and acute; context white to light umber, soft corky when fresh, corky when dry, 0.3–2 cm. thick, with a rather disagreeable odor; hymenium separated from the context by a distinct, narrow, dark-colored line; tubes short, not more than 3 mm. long, the mouths whitish or smoky, blackish when bruised, circular to somewhat angular but thick-walled and entire, averaging 4–6 to a mm., spores white, smooth, elliptical to subcylindrical, 2.6–4 x 5.3–7.2 μ.

Growing on dead wood of deciduous trees. October to December. Frequent.

Distinguished from *P. fragrans* Peck by the more circular and entire tube mouths and, in our plants at least, by the absence of the fragrant, anise-like odor. The odor is disagreeable in

the fresh plants but disappears on drying. Bresadola ascribes a subanise odor to the plant at times. The plants are, however, closely related and one may expect to find intermediate forms that are difficult to refer to either species. Thin, semi-resupinate forms are often scarcely distinguishable from *P. adustus* Willd. ex Fries. The plant is illustrated by Bresadola (Fungi Trident. *pl. 135*).

25. P. robiniophila (Murr.) Overholts, n. comb.

Trametes robiniophila Murr. N. Am. Flora **9**: 42. 1907.

Plants annual, sessile, rarely imbricate; pileus dimidiate, fleshy-tough or somewhat coriaceous when fresh, firm and rigid when dry, 3.5–10 x 4–15 x 1–4 cm., white to cinereous or yellowish, finely tomentose to glabrous, azonate or rarely subzonate or concentrically sulcate in large specimens, margin at first thick and obtuse, becoming thin and acute when mature; context white, fleshy-tough when fresh, soft and punky when dry, 0.5–3 cm. thick, usually with a sweet anise-like odor developing in herbarium specimens; tubes 0.3–1 cm. long, mouths white, often bay or brownish in dried plants, circular to angular, averaging 4–6 to a mm., the walls thick and entire; spores white, smooth, ovoid to subglobose, 5.5–7 x 7–8.5 μ.

On deciduous trees, especially *Robinia*, *Celtis*, and *Acer*. August to December. Common.

Dried plants are characterized by the tough, punky context and the sweet odor, as well as by the large size of the plant, the long tubes, the minute mouths, and the habitat. The plant was first described as a *Trametes* but it appears to belong rather to *Polyporus*.

26. P. betulinus Bull. ex Fries, Syst. Myc. **1**: 358. 1821.

Boletus betulinus Bull. Herb. Fr. *pl. 312*. 1786.

Pileus sessile or attached by a prominent lateral umbo, dimidiate to circular in outline, 3–9 x 3–15 x 1–5 cm., somewhat fleshy when young, firm and rigid when dry, glabrous, azonate, smooth, covered with a thin pellicle, margin more or less incurved, with a wide sterile band on the lower surface; context white, somewhat fleshy when fresh, soft-corky when dry, 1–3.5 cm. thick; tubes 3–8 mm. long, mouths white, circular to angular, averaging 3–4 to a mm.; hymenium at times covered by projecting setae, sometimes as much as 2 mm. long; tubes separat-

ing in a smooth layer from the context; spores (teste Murrill) white, cylindrical, curved, 4–5 μ long.

Growing only on *Betula*. Not common.

Always easily recognized by the habitat, the smooth, pelliculose surface and the inrolled, broadly sterile margin of the pileus. Good illustrations are given by Freeman (Minn. Plant Diseases *f. 126*), Hard (Mushrooms *f. 337*), White (Hymen. Conn. *pl. 37*), and Kellerman (Ohio Myc. Bul. 10: *f. 43*).

27. P. volvatus Peck, Rept. N. Y. State Mus. **27**: 98. 1875.

Plants annual, sessile or attached by a stem-like base; pileus globose or compressed-globose in form, 1–5.5 cm. broad, 1–3.5 cm. thick, somewhat coriaceous-corky when fresh, hard and firm when dry, somewhat encrusted, whitish or yellowish, sometimes tinged with red, glabrous, azonate, margin thick and rounded, extending downward and backward and forming a veil-like covering over the hymenium; context white or light colored, soft-corky, 0.2–1 cm. thick; tubes 2–5 mm. long, the mouths whitish to brownish, circular, averaging 3–4 to a mm.; the covering over the hymenium ruptures in from one to three places and allows the escape of the spores; spores (teste Peck) flesh-colored, elliptical, 5 x 7.5–9 μ.

On dead wood of coniferous trees. Rare.

An aberrant form easily recognized by the veil-like covering of the hymenium. This is persistent, being coriaceous in texture and as much as 1 mm. thick. Peck's illustration (Rept. N. Y. State Mus. **27**. *pl. 2. f. 3–6*) gives some idea as to the general form of the plant; Hard's (Mushrooms *f. 340*) is not much better. von Schrenk gives a good illustration (U. S. Dept. Agr., Div. Veg. Path. Bul. 25: *pl. 1. f. 2*).

28. P. distortus Schw. ex Fries, Elench. Fung. **1**: 79. 1828.

Boletus distortus Schw. Syn. Fung. Car. 97. 1822. *Polyporus abortivus* Peck, Bot. Gaz. **6**: 274. 1881.

Plants stipitate or substipitate, variable in form and size, sometimes with a distinct, well developed, centrally placed stipe, sometimes the whole plant distorted and the stipe rudimentary, often almost the entire surface of such forms covered with the tubes; pileus circular to irregular in outline, fleshy-tough when fresh, firm and coriaceous when dry, variable in color, whitish, grayish, tan-colored, rufescent, or brownish, vil-

lous-tomentose, soft to the touch, azonate, margin thin and acute or thick and obtuse; context white or whitish, with a firm corky layer next to the hymenium and a lighter colored, softer layer above, the whole 0.2–1 cm. thick; tubes in well developed specimens 1–6 mm. long, whitish or rufescent when bruised, mouths angular to dædaloid and irregular, averaging 1–3 to a mm.; stipe central, lateral, or wanting, rarely well developed and up to 6 cm. long, more often rudimentary and tubercular, clothed like the pileus, soft on the outside and firm within; spores white, smooth, subglobose, 5.5–8.5 μ in diameter; conidial (?) spores sometimes present, white, smooth, ovoid to elliptical, 3.3–4.2 x 5.2–7.8 μ.

Usually growing about stumps and probably always attached to buried wood. Common.

Well developed specimens of this plant will be easily recognized by the duplex context and the soft, villous pileus; abnormal specimens by their distorted appearance. The duplex context is always more easily recognized in dried specimens. According to Lloyd our plant is identical with *P. rufescens* Fries of Europe. See Lloyd, Syn. Stip. Polyp. *f. 458.*, for illustration of one form of the distorted plant.

29. P. pocula Schw. ex Berk. & Curt. Proc. Am. Acad. Arts Sci. **4**: 122. 1858.

Sphaeria pocula Schw. Jour. Acad. Nat. Sci. Phil. **5**: 7. 1825. *Enslinia pocula* Schw. ex Fries, Summ. Veg. Scand. **2**: 399. 1849.

Pileus short-stipitate, pendant from dead branches, circular in outline, 1–5 mm. in diameter, 1–3 mm. thick, coriaceous when fresh, rigid when dry, whitish to brown in color, pruinose or mealy, azonate; context coriaceous when fresh, hard when dry, less than 1 mm. thick; tubes not more than 0.5 mm. long, mouths at first appearing pruinose, whitish or brownish, circular, very minute, averaging 5–6 to a mm.; stipe dorsally attached, concolorous with and expanding into the pileus, pruinose, not more than 5 mm. long; spores (teste Murrill) globose, smooth, hyaline, 4 μ in diameter.

On dead branches, especially of *Quercus* and *Castanea*. Rare.

This is the smallest known polypore and easily identified by its size and habit of growth. It was first decribed as an asco-

mycete (*Sphaeria*) by Schweinitz and later transferred to the genus *Enslinia* (*Pyrenomycetes*) by Fries. Excellent illustrations are given by Lloyd (Myc. Notes, Polyp. Issue 3: *f. 369–70;* Syn. Stip. Polyp. *f. 443*).

30. P. brumalis Pers. ex Fries, Syst. Myc. 1: 348. 1821.
Boletus brumalis Pers. Neues Mag. Bot. 1: 107. 1794.

Pileus stipitate, circular in outline, sometimes somewhat umbilicate in the center, 1.5–5 cm. broad, 0.2–0.4 cm. thick, fleshy-tough when fresh, rigid when dry, varying in color from yellowish brown to dark brown or almost black, minutely hispid to glabrous, rarely slightly squamulose, usually azonate but at times distinctly zoned, margin thin and entire, involute when young and incurved on drying; context white or pallid, soft-fibrous when fresh, firm when dry, 2 mm. or less thick; tubes 1–3 mm. long, usually slightly decurrent, the mouths white or whitish, at first circular and thick walled, later angular and the dissepiments thinner, averaging 2–3 to a mm.; stipe central or subcentral, simple, cylindrical, grayish or brownish, minutely hispid or glabrous, 2–3 cm. long, 0.2–0.3 cm. thick; spores white, oblong, sometimes slightly curved at one end, smooth, 2.5 x 9 μ.

Growing on dead wood in the fall and early winter. Common.

P. brumalis and *P. arcularius* are closely related species that are not always easy to separate. In general the forms occurring in the early spring and summer are likely to be *P. arcularius,* while those found in autumn and often late in winter are more likely to be *P. brumalis.* Hard (Mushrooms *f. 335*) gives a good illustration of the plant.

31. P. arcularius Batsch. ex Fries, Syst. Myc. 1: 342. 1821.
Boletus arcularius Batsch. El. Fung. 97. 1783. *P. arculariformis* Murrill, Torreya 4: 151. 1904.

Pileus stipitate, circular in outline, convex to umbilicate, sometimes infundibuliform, 1–8 cm. broad, 1–4 mm. thick, fleshy-tough or coriaceous when fresh, rigid when dry, golden brown to dark brown, usually more or less squamulose, azonate, the margin usually distinctly ciliate, involute on drying; context white or pallid, fibrous-fleshy when fresh, compact-fibrous when dry, less than 2 mm. thick; tubes 1–2 mm. long, often decurrent, the mouths white, discolored on drying, angular and

often radially elongate, averaging 2 to a mm. in transverse direction and about 1 to a mm. in axial direction; stipe central or subcentral, concolorous with the pileus, fuscous-squamulose to glabrous above, often hispid at the base, 2–6 cm. long, 2–4 mm. thick; spores white, smooth, elliptical-cylindrical, usually 2–3 guttulate, 2–3 x 6–8.5 μ.

On dead wood. Common.

This species is much more common than the preceding and is distinguished from it by the lighter colored pileus, the ciliate margin, the hispid stipe base, and the larger and more alveolar tubes. Is is usually found in the spring and early summer. A small form of it with the pileus not more than 1 cm. in diameter is especially common on twigs and bits of wood during the late spring and early summer. Murrill regards this form as a distinct species and has named it *P. arculariformis* (Torreya **4**: 151). It is here maintained as a form of *P. arcularius*. This species is well represented by Hard (Mushrooms *f. 336*).

32. P. pennsylvanicus Sumstine, Jour. Myc. **13**: 137. 1907.

Pileus stipitate, circular in outline, depressed, sometimes umbilicate or somewhat infundibuliform, 4–6.5 cm. in diameter, 0.2–0.5 cm. thick, fleshy-tough, pale tan or ochraceous buff in color, with a thin cuticle, glabrous, azonate, margin thin and acute; context white, soft and watery when fresh, with a sweet acid odor, rather fragile when dry, 2–4 mm. thick; tubes white at first, discolored on drying, long decurrent on the stipe, 2–4 mm. long, mouths angular, thin walled, large, somewhat longer in the radial direction, 1–2 mm. long, 0.5 to 1 mm. wide; stipe central or excentric, whitish, glabrous, expanding above, 2–3 cm. long, 0.4–1 cm. thick; spores white, smooth, oblong-elliptical or fusoid, 4.2–5.7 x 10–14 μ, often once to several times guttulate.

Growing on old logs in July and August. Frequent.

The above description is drawn from notes and specimens from two collections made at Oxford, Ohio, one in August, 1910, and the other in July, 1911. The odor of the fresh plant is described by the author as "nitrous". The large angular pores ally the species with *P. arcularius* Batsch. ex Fries and with *Favolus canadensis* Klotzsch. From the former it is easily separated by the much larger spores and from the latter by

the well developed stipe with the decurrent tubes, the usually umbilicate pileus, and the friable context when dry. Possibly it should be referred to *P. Rostkowii* Fr. or to *P. pallidus* Schulz. & Kalchbr., both of which some regard as being small scaleless forms of *P. squamosus* Huds. ex Fries. The spores agree well with those of *P. squamosus*, but although it can be shown to be related to that species, it is worthy of a distinct name.

33. P. squamosus Huds. ex Fries, Syst. Myc. 1: 343. 1821.
Boletus squamosus Huds. Fl. Angl. 626. 1798. [2nd ed.]

Pileus short-stipitate or almost sessile, dimidiate to reniform in outline, 6–25 cm. in diameter, 0.5–4 cm. thick, fleshy when fresh, firm and rigid when dry, whitish to dingy yellowish or brownish, clothed, especially toward the center, with large, appressed, brownish scales often concentrically arranged, azonate, margin thin and acute; context white, tough, soft-corky when dry, 0.5–3.5 cm. thick; tubes 2–8 mm. long, decurrent, the mouths white or yellowish, large and angular, 1–2.5 mm. in diameter; stipe lateral, often rudimentary, black at the base, reticulate above by the decurrent pores, 1–5 cm. long, 1 cm. or more thick.

Growing on injured or diseased deciduous trees. Rare.

Lloyd gives the spores as "oblong, 5–6 x 12–15 μ, hyaline, smooth." Easily recognized by the large pores and the large, appressed, brownish scales. The plant is well illustrated by Bresadola (Fung. Trident. *pl. 133*), Freeman (Minn. Pl. Diseas. *f. 125*), Lloyd (Photograph. *pl. 5*), and Hard (Mushrooms *f. 325*).

34. P. picipes Fries, Syst. Myc. 1: 353. 1821.
P. fissus Berk. Hooker's Lond. Jour. Bot. **6**: 318. 1847.

Pileus stipitate, circular to reniform in outline, convex or plane, when older usually becoming depressed or somewhat infundibuliform, 4–20 cm. broad, 0.1–0.8 cm. thick, tough and leathery when fresh, very rigid and brittle when dry, sometimes yellowish brown but usually dark chestnut-brown to reddish brown, usually lighter in color towards the margin, azonate, margin very thin, usually wavy and often lobed; context white to somewhat ochraceous, leathery when fresh, firm when dry, 1–7 mm. thick; tubes not more than 2 mm. long, decurrent on

the stipe, the mouths white to brownish in color, circular to angular, very minute, invisible to the unaided eye, averaging 5–7 to a mm.; stipe central to lateral, dark brown or black on the lower half, glabrous, 1–6 cm. long, 0.4–1.5 cm. thick.

On stumps and logs late in autumn. Common.

The combination of black stipe base and minute pores characterizes this and the next species. The two are separated mainly on point of size. Murrill describes this plant under the name of *P. fissus* Berk., which was originally described from specimens collected in Ohio. Patouillard (Tab. Fung. No. 136) says the spores are ovoid. Lloyd now considers this plant to be a form of *P. varius* Fries, of Europe. A good illustration of our plant will be found in Hard, Mushrooms *f. 319.*

35. P. elegans Bull. ex Fries, Epicr. Syst. Myc. 440. 1838.
Boletus elegans Bull. Herb. Fr. *pl. 46.* 1780.

Pileus stipitate, circular to reniform in outline, convexo-plane or depressed, 1.5–7 cm. in diameter, 0.2–1 cm. thick, leathery when fresh, rigid and firm when dry, pale ochraceous to dull orange-color, pruinose to glabrous, azonate, the margin rather thin, often radiate-striate, even or undulate; context white to light ochraceous, tough when fresh, soft corky when dry, 1–6 mm. thick; tubes 1–3 mm. long, decurrent on the stipe, the mouth whitish to pallid, circular to angular, averaging 4–5 to a mm.; stipe central, excentric or lateral, slender, black at the base, light colored above, pruinose or glabrous, 1-8 cm. long, 0.2–0.6 cm. thick.

On dead wood late in autumn. Not common.

Spores were not obtained from the writer's specimens. Murrill gives them as "oblong, smooth, hyaline, 7–8 x 3–3.5μ." The species is closely related to *P. picipes* Fries but is separated from it by the smaller size and the uniform ochraceous color of the pileus that never takes on the darker chestnut shades assumed by *P. picipes.* Bulliard (Herb. Fr. *pl. 124*) gives an excellent illustration of the plant under the name of *Boletus nummularius* Bull.

36. P. radicatus Schw. Trans. Am. Phil. Soc. II. **4**: 155. 1832.

P. Morgani Peck, Ann. Rept. N. Y. State Mus. **32**: 34. 1879.

Pileus stipitate, circular in outline, 3.5–20 cm. broad, 0.3–0.8

cm. thick, fleshy or fleshy-tough when fresh, more or less friable when dry, yellowish brown or darker, finely tomentose or fibrillose-scaly, often becoming glabrous, azonate; margin thin and acute, often involute on drying; context white or light yellowish, soft and spongy, 2–6 mm. thick; tubes 1–5 mm. long, decurrent on the stipe, the mouths white or brownish on drying, circular to angular and irregular, averaging 2–3 to a mm.; stipe central, simple or rarely branching once or twice, yellowish or brownish, prolonged below into a long, black, rooting base, velvety or rough-squamulose above, 6–15 cm. long, 0.5 to 2 cm. thick; spores white, smooth, ovoid-elliptical, 6–8 x 12–15 μ.

Growing on the ground, sometimes around stumps, and probably attached to buried wood. Common.

This plant is always easily recognized by the black and radicating base of the stem. The type specimens of *P. Morgani* Peck were collected in Ohio by Morgan. For illustrations see Hard, Mushrooms *f. 329.*, Lloyd, Syn. Sec. Ovinus *f.* 508; Syn. Stip. Polyp. *f. 465.*, and Ohio Myc. Bull. 11: *f. 46.*

37. P. flavovirens Berk. & Curt. Grev. 1: 38. 1872.

Pileus stipitate, circular to irregular in outline, 4–10 cm. broad, 0.3–0.8 cm. thick, soft and fleshy when fresh, rigid but friable when dry, yellowish green or yellowish brown in color, the surface often cracked and areolate and the flesh showing yellowish in the cracks, slightly tomentose or glabrous, azonate, the margin thin and acute; context white or yellow, fleshy when fresh, soft and friable when dry, 1–4 mm. thick; tubes 1–5 mm. long, decurrent on the stipe, the mouths white or yellowish, sometimes reddish on drying, circular to angular, averaging 1–3 to a mm.; stipe simple or branched, usually excentric but sometimes central, often irregular in form, whitish or yellowish in color, 3–6 cm. long, 1–1.5 cm. thick; spores white, smooth, globose, or subglobose, 3–4.7 μ in diameter.

Growing on the ground in deciduous woods. Frequent in July and August.

A species easily recognized by the color of the pileus. The plant is fairly well represented by Hard (Mushrooms *f. 327*), and by Lloyd (Syn. Sect. Ovinus *f. 501*). According to Lloyd *P. cristatus* Pers. of Europe is not different from our plant. Murrill lists it under the name of *Grifola poripes* Fries ex Murr.

38. P. umbellatus Pers. ex Fries, Syst. Myc. 1: 354. 1821.
Boletus umbellatus Pers. Syn. Fung. 519. 1801.

Plants stipitate, 7–20 cm. in diameter, the stipe branching repeatedly and giving rise to many centrally attached pileoli which are circular in outline, 1–4 cm. broad, less than 5 mm. thick, fleshy in texture when fresh, rigid when dry, whitish to smoky brown in color, fibrillose or glabrous, azonate; margin thin, acute, entire; context white, fleshy or fleshy-tough, rather brittle when dry, usually not more than 1 mm. thick; tubes less than 2 mm. long, decurrent on the stipe branches, the mouths white, angular, averaging 2–4 to a mm.; stipe compound, the branches cylindrical in form, central or subcentral, white, usually entirely covered with the decurrent tubes; spores white, oblong-elliptic, smooth, 2.3–3.5 x 7–9.4 μ.

Growing about the bases of stumps or trees, especially of *Quercus*. Rare.

Easily distinguished from its allies by the more regular and cylindrical stipe branches, the small and centrally attached pilei which are more or less circular in outline, and by the oblong-elliptic spores. Murrill describes it as *Grifola ramosissima* Scop. ex Murr. The plant is well illustrated by Lloyd (Syn. Stip. Polyp. *f. 450*), Hard (Mushrooms *f. 320*), and Atkinson (Mushrooms *f. 183*).

39. P. frondosus Dicks. ex Fries, Syst. Myc. 1: 355. 1821.
Boletus frondosus Dickson, Fasc. Pl. Crypt. Brit. 1: 18. 1785.

Plant stipitate, the stipe many times branching and giving rise to numerous overlapping pileoli, the whole plant forming a more or less globose mass often as much as 40 cm. in diameter; pileoli flabelliform or spathulate in outline, 2–7 cm. broad, 2–5 mm. thick, fleshy-tough when fresh, rigid when dry, grayish to mouse-colored, glabrous or minutely tomentose, azonate, the margin thin and acute; context white or whitish, fleshy-tough when fresh, fragile when dry, not more than 2 mm. thick; tubes 2–3 mm. long, decurrent on the stipe, the mouths white, angular or irregular, averaging 1–3 to a mm.; stipe compound, short and thick; spores white, smooth, ovoid to elliptical, 4.5–6 x 6–9 μ.

Usually found at the bases of trees or stumps, preferably of *Quercus* and *Ulmus*. Common in late fall.

From *P. Berkeleyi* Fries and *P. giganteus* Fries this species is separated by the numerous small pileoli which in those species are large and few in number. The irregular stipe-branches and the more spathulate pileoli separate it from *P. umbellatus* Fries in which the stipe branches are cylindrical and the pileoli centrally attached and consequently more nearly circular in outline. The plant is illustrated in Atkinson, Mushrooms *f. 181–82.*, Hard, Mushrooms *f. 321.*, and McIlvaine, Am. Fungi *pl. 128.*

40. P. giganteus Pers. ex Fries, Syst. Myc. **1** : 356. 1821.

Boletus giganteus Pers. Syn. Fung. 521. 1801. *Grifola Sumstinei* Murrill, Bull. Torr. Club **31**: 335. 1904.

Plants composed of a few broad pileoli, 6–15 cm. in diameter and less than 0.5 cm. thick, dimidiate to flabelliform or spathulate in outline, fleshy-fibrous when fresh, more rigid when dry, grayish to brown, often black when dried—especially on the margin—,usually somewhat tomentose or fibrillose, azonate or subzonate, margin very thin and acute, often lobed, involute on drying; context white, fibrous, tough, 1–3 mm. thick; tubes 1–3 mm. long, at first white but blackish where bruised and on drying, the mouth angular to irregular, often torn, averaging 5–7 to a mm.; stipe short and thick; spores white, smooth, globose, 4–6 μ broad.

Growing on the ground around stumps. Frequent.

Separated from *P. Berkeleyi* Fries by the smooth spores; from *P. umbellatus* Pers. ex Fries, and *P. frondosus* Fries, by the much larger and fewer pileoli, and distinct from all of these in the blackening of the margin or of the entire pileus and hymenium when bruised or in drying. In the 'North American Flora' it is described under the name of *Grifola Sumstinei* Murr. In this country it has always been held to be the same as the European plant *P. giganteus* Fries, and European specimens recently received from Bresadola confirm this view. A very good illustration will be found in Bresadola, Fungi Tridenti *pl. 134.*, and in Boudier, Ic. Myc. **1**: *pl. 153.* To the writer's knowledge it has not been illustrated in American mycology.

41. P. Berkeleyi Fries, Nov. Sym. 40. 1851.

P. anax Berk. Grev. **12**: 37. 1882.

Pileus stipitate, the stipe sometimes branching and giving

rise to from 2 to 4 pileoli, sometimes simple with but one large pileus; pileoli fleshy-tough when fresh, becoming rigid on drying, more or less circular in outline, 6–15 cm. broad, 0.3–1.5 cm. thick, light colored, whitish to yellowish, slightly tomentose or glabrous, azonate or obscurely zoned; margin rather thin, often lobed; context white, fleshy-tough, fragile when dry, 0.3–2 cm. thick; tubes 2–8 mm. long, decurrent on the stipe; mouths white or whitish, large and irregular, averaging 0.5–2 mm. in diameter; stipe short and thick, more or less tubercular, whitish in color, 4–7 cm. long, 3–5 cm. thick; spores white, minutely echinulate, globose, 5.6–8.4 μ in diameter.

Growing at the bases of trees and stumps, especially of *Quercus*. Frequent.

This is one of the largest of our species and is easily distinguished from all of its allies by the echinulate spores. Morgan's description of *P. anax* Berk. applies to *P. frondosus* Fries and not to *P. Berkeleyi* for which *P. anax* is a synonym. (See Lloyd, Mycological Notes 27: 341–342.) The plant is well represented by the following illustrations: Lloyd, Photogr. *pl. 9–10;* Myc. Notes Polyp. Iss. 3: *f. 362–63.*, and Hard, Mushrooms *f. 323* and *pl. 45.*

42. P. sulphureus Bull. ex Fries, Syst. Myc. **1**: 357. 1821.

Boletus sulphureus Bull. Herb. Fr. *pl. 429.* 1788. *P. cincinnatus* Morgan, Jour. Cinc. Soc. Nat. Hist. **8**: 97. 1885.

Plants annual, often attenuate at the base and appearing substipitate, imbricate; pileus dimidiate to flabelliform in outline, 5–20 x 4–12 x 0.5–2.5 cm., fleshy and watery when young, becoming firm when old, yellowish to bright orange-colored, sometimes fading with age, finely tomentose to glabrous, azonate or with broad colored zones, the margin thin and acute, sometimes lobed; context white or light yellow, fleshy when fresh, rather soft and friable when dry, 0.4–2 cm. thick; tubes 1–4 mm. long, the mouths bright sulphur-yellow, sometimes whitish or dull yellow with age or on drying, angular, averaging 2–4 to a mm.; spores white, smooth, ovoid to subglobose, 4–5 x 5.5–7 μ.

Growing on trunks and stumps of deciduous trees. Common.

Specimens usually change color on drying and most of the red color of the pileus is lost. The bright yellow of the hyme-

nium may or may not persist. The best colored representation of the fungus is that given by Rostkowius in Sturm, Deutschl. Flora 4: *pl. 20.* The plant is widely distributed and well known and has figured largely in American mycology. The following illustrations will aid in determinations: Atkinson, Mushrooms *f. 184–85.*, Duggar, Fung. Dis. Pl. *f. 226.*, Hard, Mushrooms *f. 326.*, and von Schrenk, U. S. Dept. Agr., Div. Veg. Path. Bul. 25: *pl. 11. f. 1–4.*

43. P. Pilotae Schw. Trans. Am. Phil. Soc. II. 4: 157. 1832.

P. hypococcineus Berk. Lond. Jour. Bot. **6**: 319. 1847.

Plants annual, sessile; pileus dimidiate, often subungulate, 5–12 x 6–15 x 1–5 cm., soft coriaceous or corky, buff or orange-colored, becoming whitish on drying, minutely tomentose or glabrous, azonate, margin usually obtuse; context pale buff, becoming carneous when dry, fibrous, sometimes very hard when dry, strongly zonate, 0.7–2 cm. thick; tubes 0.5–2 cm. long, the mouths orange-colored, becoming brownish on drying, angular, averaging 3–5 to a mm.; spores (teste Murrill) smooth, hyaline, 3–4 x 2–3 μ.

On dead wood of *Quercus* and *Castanea.* Rare.

Easily distinguished from other species with a predominance of red or orange colors by the thick pileus and the long tubes. The plant is said to emit a strong odor when growing. The type specimens of *P. hypococcineus* Berk. were collected in Ohio by Lea. *P. castanophilus* Atk., described from North Carolina, is said to be the same plant.

44. P. sanguineus L. ex Fries, Syst. Myc. 1: 371. 1821.

Boletus sanguineus L. Sp. Plant. 1646. 1762. [2nd ed.]

Plants annual, sessile; pileus dimidiate to flabelliform, 2–5 x 2–8 x 0.2–0.5 cm., coriaceous, bright red, finely tomentose to glabrous, often zonate, the margin very thin and acute; context red or yellowish red, soft and floccose, scarcely more than 2 mm. thick; tubes 0.5–1.5 mm. long, the mouths red, more or less angular or circular when young, averaging 2–4 to a mm.; pileus often attached by an attenuate base and then appearing substipitate.

On dead wood of deciduous trees. September to December. Rare.

The species is distinguished from the following one by the much thinner pileus and the marked tendency to appear substipitate. Otherwise it scarcely differs, and intermediate forms are found that are difficult to place satisfactorily It is usually considered to be a southern species, but Hard reports finding it in Ohio. His specimens were determined by Peck.

45. **P. cinnabarinus** Jacq. ex Fries, Syst. Myc. 1: 371. 1821.
Boletus cinnabarinus Jacq. Fl. Austr. 4: 2. 1776.

Plants annual, rarely reviving, sessile or effused-reflexed; pileus dimidiate or reniform, 2–6 x 2–10 x 0.5–2 cm., tough and leathery when fresh, more rigid when dry, orange-colored to cinnabar-red, often becoming paler or almost white with age, compactly tomentose or glabrous, usually azonate, margin thin or thick, acute; context red or yellowish red, floccose-fibrous to soft-corky, always zonate, 0.4–1.5 cm. thick; tubes 1–4 mm. long, the mouths cinnabar-red, circular then angular and sometimes somewhat sinuous, averaging 2–4 to a mm.; spores white, smooth, oblong, 2–2.5 x 4.5–5.5 μ.

On dead wood of all kinds. September to December. Common.

The prevailing deep red color of both pileus and hymenium separates this species from all others of the genus except *P. sanguineus* Fries, from which this species differs only in being thicker and in having the context more strongly zoned. *P. cinnabarinus* is a northern species and much more common in Ohio than is *P. sanguineus.*

46. **P. resinosus** Schrad. ex Fries, Syst. Myc. 1: 361. 1821.
Boletus resinosus Schrad. Spic. Fl. Ger. 171. 1794.

Plants annual, sessile or decurrent, more or less imbricate; pileus dimidiate, 5–15 x 7–25 x 0.8–2.5 cm., somewhat fleshy and full of water when young, firmer when mature and soft-corky on drying, velvety-tomentose to glabrous, sulcate or with a few broad, colored zones, margin at first thick and somewhat obtuse, becoming thinner and acute; context pallid to light brown, fleshy and watery when young, soft-corky when dry, 0.5–2 cm. thick; tubes 1–6 mm. long, the mouths white to pallid, changing to a darker color on drying, circular to angular, averaging 4–6 to a mm.; spores white, smooth, cylindrical, curved, 1.2–2 x 5–6.3 μ.

On old logs and stumps in October and November. Common.

Distinguished by the brown pileus and the light brown, almost whitish context. For illustration see Hard, Mushrooms *f. 331*.

47. P. nidulans Fries, Syst. Myc. 1:362. 1821.

Plants sessile or effused-reflexed; pileus dimidiate, 1.5–6 x 2–8 x 0.5–2 cm., very soft, spongy, and full of water when fresh, firm and friable when dry, umber to cinnamon or tawny brown, finely villous-tomentose to glabrous, azonate, margin thin and acute, purplish or reddish where bruised; context concolorous with the pileus, sometimes with a darker layer next to the hymenium, soft and watery when fresh, cheesy and friable when dry, 2–8 mm. thick; tubes 2–7 mm. long, mouths hoary when young, yellowish or reddish brown when mature, angular or sinuous, averaging 3–4 to a mm.; spores white, smooth, globose or subglobose, 2–3.5 μ in diameter.

On dead wood of deciduous trees, especially *Quercus*. June to September. Not common.

Distinguished by the uniform umber brown color of the whole plant, the soft and watery context, etc.

48. P. gilvus Schw. ex Fries, Elench. Fung. 1: 104. 1828.

Boletus gilvus Schw. Syn. Fung. Car. 96. 1822.

Plants annual or reviving for two or three years, sessile or effused-reflexed, often imbricate; pileus dimidiate, 1–7 x 2–12 x 0.2–2 cm., leathery or corky when fresh, woody and rigid when dry, yellowish brown or reddish brown, in very young stages covered by a purplish, villous pubescence, otherwise glabrous, usually rough, more or less zonate, margin thin and acute; context yellowish brown, soft-corky to woody, 0.1–1.3 cm. thick; tubes 1–5 mm. long, the mouths reddish brown or darker, circular, then angular, averaging 6–8 to a mm., the walls rather thick and entire; spores white, smooth, oblong-ellipsoid, 3–4 x 5–6 μ.

On dead wood of all kinds. July to December. Common.

Closely related to *P. radiatus* Sow. ex Fries and *P. cuticularis* Bull. ex Fries, but distinct in the white spores, the lighter colored surface and the more woody context. *P. isidiodes* Schw. as reported by Lea belongs here.

49. P. radiatus Sow. ex Fries, Syst. Myc. 1: 369. 1821.
Boletus radiatus Sow. Eng. Fungi *pl. 196*. 1799.

Plants annual, sessile or decurrent; pileus dimidiate or flabelliform and attached by an attenuate base, 2–5 x 2–7 x 0.3–2 cm., firm and rigid, yellowish brown or rust-colored, velvety to glabrous, sometimes conspicuously zonate, sometimes azonate, margin thin or thick, acute; context yellowish to rusty brown, corky and somewhat friable, 2–5 mm. thick; tubes 1–8 mm. long, the mouths grayish umber to rusty red, circular, then angular, averaging 4–5 to a mm.; spores (teste Bresadola) yellowish, elliptical, 3.5–4.5 x 5.5–6.5 μ.

Growing commonly on *Betula* and *Alnus*. Rare.

A species intermediate between *P. gilvus* Schw. ex Fries, and *P. cuticularis* Bull. ex Fries, distinguished from the former by the habitat, the brighter color and the smoother surface of the pileus, and by the colored spores, and from the latter chiefly in the habitat. The species was reported from Ohio by Lea but I have not examined the plants.

50. P. cuticularis Bull. ex Fries, Syst. Myc. 1: 363. 1821.
Boletus cuticularis Bull. Herb. Fr. *pl. 462*. 1809.

Plants annual, sessile, often imbricate; pileus dimidiate or flabelliform and attached by an attenuate base, 3–7 x 3.5–10 x 0.3–1 cm., spongy and fleshy-tough when fresh, leathery to rigid when dry, yellowish brown to rusty brown, compact wooly-tomentose, becoming fibrillose or almost glabrous, sometimes subzonate on the margin, margin thin, acute, often inflexed; context yellowish brown or rust-colored, tough and watery when fresh, distinctly fibrous, 2–7 mm. thick; tubes 2–7 mm. long, the mouths hoary brown to rust-colored, angular, averaging 3–5 to a mm.; spores yellowish brown, smooth, subglobose to broadly elliptical, 4.2–5.7 x 5.5–7 μ.

On dead wood of deciduous trees. August to November. Common.

This species is very closely related to *P. radiatus* Sow. ex Fries, but Ohio plants may be distinguished from that species by the habitat, the thicker and larger pileus, and by the more tomentose and spongy surface. *P. perplexus* Peck, the types of which have been destroyed, is thought by some to be this species and our plants are frequently referred to it.

51. P. hispidus Bull. ex Fries, Syst. Myc. 1:362. 1821.
Boletus hispidus Bull. Herb. Fr. *pl. 210*. 1791. *Polyporus endocrocinus* Berk. Hooker's Lond. Jour. Bot. **6**:320. 1847.

Plants annual, sessile, sometimes imbricate; pileus dimidiate, 6–20 x 9–25 x 2–6 cm., spongy and watery when fresh, firm and rigid when dry, yellowish brown to rusty red, soft from the covering of the dense hirsute or hispid tomentum or pubescence, azonate, margin thick or thin, obtuse or acute; context usually light yellowish brown above and dark reddish brown next to the hymenium, fibrous, firm when dry, 1–5 cm. thick; tubes 0.6–1.5 cm. long, mouths yellowish brown becoming darker where bruised, circular, then angular, averaging 2–4 to a mm.; spores yellowish brown, smooth, broadly ovoid to ellipsoid, 6.5–7 x 7–9.5 μ.

On living trunks of deciduous trees. September to December. Rare.

Much larger than *P. cuticularis* Bull. ex Fries, and *P. radiatus* Sow. ex Fries, and expecially distinct by the hirsute or hispid pubescence. In point of size it more nearly approaches *P. dryadeus* Pers. ex Fries, and *P. dryophilus* Berk., but easily distinguished from them by the pubescence.

52. P. dryadeus Pers. ex Fries, Syst. Myc. 1: 374. 1821.
Boletus dryadeus Pers. Obs. Myc. 3. 1799.

Plants sessile; pileus dimidiate, applanate, 6–30 x 8–35 x 2–6 cm., spongy and watery when fresh, more or less corky or woody when dry, grayish brown to dark brown or black in old specimens, glabrous, azonate, margin thick and obtuse, distilling drops of water when young and growing; context umber-brown to rust-colored, subshining when dry, soft and watery, corky or woody on drying, 1.5–4 cm. thick; tubes 0.3–2 cm. long, mouths grayish brown, darker on drying, circular, then angular, averaging 3–5 to a mm.; spores (teste Bresadola) globose or subangular, smooth, yellowish, 8–9 x 7–8 μ.

On living trunks of *Quercus*. September to November. Rare.

Very closely related to *P. dryophilus* Berk., and probably the two have been confused in this country. *P. dryadeus* is usually considered to be a more applanate form and much larger than *P. dryophilus*. There is also said to be a decided difference

in spore color in the two plants, *P. dryadeus* having much paler spores than *P. dryophilus*, but for this I cannot vouch. So far as known, *P. dryadeus* has not been collected in Ohio but the species has been reported from Michigan and Kentucky. Lloyd (Myc. Notes 36. *f. 383*) gives an illustration.

53. P. dryophilus Berk. Hooker's Lond. Jour. Bot. **6**:321. 1847.

Plants annual, sessile; pileus dimidiate, often ungulate, 3–12 x 7–20 x 1–10 cm., rather rigid, grayish brown, to reddish brown, scabrous with an innate, ferruginous pubescence, azonate or subzonate, margin thick and obtuse; context cinnamon or rusty brown, subshining, corky to hard and woody; tubes 0.3–2.5 cm. long, ferruginous-yellow within, the mouths cinnamon-brown, angular, averaging 2–3 to a mm.; spores ferruginous, smooth, ellipsoid to subglobose, 5 x 6.5 μ.

On living *Quercus* and on logs. August to November. Rare.

This species was originally described from specimens collected at Waynesville, Ohio, by Lea. To the description as given in Lea's catalogue the following note was added: "Nearly allied to *Polyporus dryadeus*, but a smaller, more rigid species with larger, differently colored pores. It has also much resemblance to *P. gilvus*."

54. P. Schweinitzii Fries, Syst. Myc. **1**:351. 1821.

Plants stipitate or sessile; pileus circular to dimidiate, 5–15 cm. broad, 0.5–1.5 cm. thick, spongy to soft-corky when fresh, firm, rigid, and sometimes friable when dry, ochraceous to orange-colored or brown in mature specimens, strigose-tomentose to almost glabrous, usually more or less zonate, margin thin or thick, acute; context yellowish to reddish brown, spongy when fresh, usually friable when dry, 0.2–1 cm. thick; tubes 1–6 mm. long, the mouths yellowish, darker when bruised and sometimes dark brown on drying, circular to angular and soon irregular, averaging 1–3 to a mm.; stipe present and well developed or entirely absent, central or excentric, agreeing in color, pubescence and consistency with the pileus, 0–6 cm. long, 1–2 cm. thick; spores (teste Lloyd) white, elliptical, smooth, 4 x 6 μ.

Growing on or about *Pinus*. Autumn. Rare.

This species is a very variable one, yet quite distinct in habi-

tat, consistency, pubescence, color, etc. It is known in Ohio only from a collection made at Cincinnati (now in the Lloyd Museum) by Mr. Wm. Holden. For illustrations see Lloyd, Myc. Notes, Polyp. Issue 1: *f. 208.*, aud von Schrenk, U. S. Dept. Agr., Div. Veg. Path. Bul. 25: *pl. 1. f. 1., pl. 2.*

55. P. circinatus Fries, Monogr. Hymen. Suec. **2**: 268. 1863.

Pileus stipitate or substipitate, circular to spathulate or flabelliform, convex to depressed, 3–9 cm. broad, 0.3–1 cm. thick, rather soft when fresh, firm when dry, yellowish to umber-brown, tomentose to velvety, azonate or subzonate, margin rather thin, acute; context yellowish to cinnamon-brown, duplex, soft and spongy above, firm next to the tubes, 1–6 mm. thick; tubes 1.5–4 mm. long, the mouths whitish to cinnamon, subcircular to angular, averaging 2–4 to a mm.; stipe sometimes rudimentary, usually lateral or excentric, fulvous to dark brown, tomentose, soft, up to 5 cm. long, 0.5–1.5 cm. thick; spores (teste Lloyd) pale color, 3 x 5 μ.

In coniferous and deciduous woods.

The species has not been reported from Ohio. It is distinguished by the duplex character of the context and by the poor development of a stipe. It is a question whether it is distinct from *P. tomentosus* Fr. Certainly *P. dualis* Peck is the same plant. Lloyd regards American plants in which the context is always duplex as belonging under *P. circinatus* Fries, and European plants with a uniform context as *P. tomentosus* Fries. The plant is illustrated by Lloyd (Myc. Notes Polyp. Issue *f. 198–99*).

56. P. obesus (Ellis & Ev.) Overholts, n. comb.

Polystictus obesus Ellis & Ev. Bull. Torr. Bot. Club **24**: 125. 1897.

Stipitate. Stipe central, spongy, velutinous, dark cinnamon, 4–6 cm. high, 0.5–1.5 cm. thick above, enlarged below to 1–3 cm.; pileus convex then depressed in the center, obconical at first with the margin obtuse, then spreading out with the margin acute, color lighter than that of the stipe, yellowish cinnamon, surface uneven, subcolliculose, not zonate, 4–6 cm. across; pores irregular, short (1 mm.), at first round with margins thick, finally irregular and subsinuous, 0.5–1 mm. across, margins acute; spores elliptical, ferruginous, 7-8 x 4–5 μ.

On the ground, in contact with and partly attached to decaying pine limbs partly buried in the soil. (The above description is according to Ellis and Everhardt, Bull. Torr. Bot. Club. **24**: 125. 1897.)

Distinguished from the next three species by the greater thickness of the pileus and stipe. From *P. circinatus* Fries, it is separated by the absence of a duplex context and by the slightly smaller pores. The plant is recorded by Morgan as *P. Montagnei* Fries, but according to Lloyd the record is based on plants collected in Canada by Dearness. It is listed in Lea's catalogue under the same name.

57. P. focicola Berk. & Curt. Jour. Linn. Soc. Bot. **10**: 305. 1868.

Pileus stipitate, circular in outline, convex-depressed to umbilicate, 2–4 cm. broad, 1–6 mm. thick, coriaceous when fresh, rigid when dry, grayish brown to cinnamon, finely tomentose, striate, zonate, margin thin and acute; context cinnamon-brown, fibrous, less than 0.5 mm. thick; tubes 1–6 mm. long, the mouths angular or irregular, cinnamon to rusty brown, averaging 1 mm. or more in diameter; stipe central, light to dark brown, minutely velvety, 1.5–3 cm. long, 2–4 mm. thick; spores (teste Lloyd) pale colored, smooth, elliptical, 5 x 10 μ.

On burned earth in woods. July to November. Rare.

The species differs from *P. perennis* L. ex Fries only in the much larger pores. The plants were reported by Lea as *P. connatus* Schw. and by Morgan as *P. parvulus* Klotzsch. The plant is well illustrated by Lloyd (Myc. Notes Polyp. Issue 1: *f. 203–4*).

58. P. perennis L. ex Fries, Syst. Myc. **1**: 350. 1821.

Boletus perennis L. Sp. Plant. 1177. 1753.

Pileus stipitate, circular in outline, convex-depressed to umbilicate, 1.5–7 cm. broad, 1–3 mm. thick, coriaceous, rigid when dry, grayish brown to cinnamon or rust-colored but never silky, finely tomentose, zonate, margin thin and acute; context cinnamon-brown, fibrous, less than 1 mm. thick; tubes 1–2.5 mm. long, the mouths grayish to cinnamon, angular, averaging 2–4 to a mm.; stipe central or subcentral, cylindrical, concolorous with the pileus, velvety, 1.5–5 cm. long, 1–6 mm. thick; spores (teste Lloyd) pale colored, 4–5 x 8–10 μ.

Growing on burned earth. July to November. Not common.

The plant closely resembles the next species but is separated from it by the habitat and the dull cinnamon or cinnamon-gray color of the zonate pileus. *Polystictus proliferus* Lloyd is said by its author to be a form of this species. It was collected near Cleveland. This species is illustrated by Atkinson (Mushrooms *f. 187*), Hard (Mushrooms *f. 346*), and Lloyd (Myc. Notes Polyp. Issue 1: *f. 201*).

59. P. cinnamomeus Jacq. ex. Fries, Epicr. Syst. Myc. 429. 1838.

Boletus cinnamomeus Jacq. Coll. Bot. etc. **1**: 116. 1786. *P. subsericeus* Peck, Ann. Rept. N. Y. State Mus. **33**: 37. 1880.

Pileus stipitate, circular in outline, convex-depressed to umbilicate, 1–5 cm. broad, 1–3 mm. thick, pliant and tough, bright cinnamon-rufous to bright amber-brown, silky fibrillose, the fibrils sometimes suberect towards the center of the pileus, silky striate, sometimes zonate, margin thin and acute; context cinnamon or rusty brown, fibrous, less than 0.5 mm. thick; tubes not more than 2 mm. long, the mouths rufous-cinnamon, angular, averaging 2–4 to a mm.; stipe central, cylindrical, concolorous with the pileus, velvety to villous, 1–4 cm. long, 1–3 mm. thick; spores (teste Lloyd) pale colored, elliptical, smooth, 5–6 x 7–10 μ.

Most frequently on clay banks, usually among moss. July to September. Not common.

Distinguished from *P. circinatus* Fries, and *P. obesus* Ellis & Ev. by the very thin context; from *P. perennis* L. ex Fries by the silky pileus and the habitat; from *P. focicola* Berk. & Curt. by the much smaller pores. For illustrations see Lloyd, Myc. Notes Polyp. Issue 1: *f. 200.*, and Bresadola, Fungi Trid. *pl. 99.*

60. P. lucidus Leyss. ex Fries, Syst. Myc. **1**: 353. 1821.

Boletus lucidus Leyss. Flora Halensis 300. 1783. [2nd ed.] *Ganoderma sessile* Murr. Bull. Torr. Bot. Club **29**: 604. 1902. *Ganoderma subperforatum* Atk. Bot. Gaz. **46**: 337. 1908.

Plants stipitate or sessile, annual; pileus dimidiate or reniform in outline, 3-12 x 3.5-20 x 0.4-2.5 cm., coriaceous-corky when fresh, corky or woody when dry, the upper surface covered by an encrusting persistent layer of deep reddish chestnut varn-

ish, often wrinkled, glabrous or pruinose from a coating of brown conidial (?) spores, zonate or concentrically sulcate, the margin thin and acute, sometimes lobed; context whitish to light brown, sometimes separated into an upper, light colored, soft layer, and a lower darker and firmer layer, but often uniform in color and texture, 0.2–1.5 cm. thick; tubes 0.3–1.5 cm. long, not decurrent, the mouths white or umber, darker when bruised, circular to angular, averaging 3–5 to a mm.; the hymenium often with red-varnished patches on which no tubes are produced; stipe often entirely absent, lateral when present, covered like the pileus, 1–10 cm. long, 0.5–1 cm. thick; spores yellowish brown, smooth or apparently slightly verrucose, ovoid with a truncate base, 5–6.3 x 9.4–11μ.

On stumps and trunks of dead or injured deciduous trees. Common.

The variation in the pileus from stipitate to sessile may be confusing at first, but the deep chestnut-red color, not changing to yellowish as in the next species, will usually be found to be the distinguishing character of the species. The plant is described by Murrill under the name of *Ganoderma sessile* Murr. Atkinson has described a new species of *Ganoderma* from Ohio under the name of *G. subperforatum*. At the writer's request Professor Atkinson very kindly sent the type collection for examination. Under the ordinary high power of the microscope the spores of both *P. lucidus* and *G. subperforatum* appear to be practically smooth. By the use of the oil-immersion lens varying degrees of apparent echinulation are to be made out in the ordinary forms of *P. lucidus* while in the type collection of *G. subperforatum* the spores do not have that appearance, although Professor Atkinson states that by first boiling the spores in potassium hydroxide solution the perforations in the spore walls are faintly visible. I am convinced, however, that the echinulate appearance when present is not due to projections on the outer wall, but, as Atkinson has said, to perforations in the inner spore wall. An examination of the dozen or more collections of *P. lucidus* in my own herbarium have given evidence of a great variability in this character. Since *G. subperforatum* is not otherwise to be distinguished from *P. lucidus*, it has seemed best to consider the name as a synonym in this paper. Even

were the character constant one might well question the advisability of separating the species on a character that requires the use of the oil-immersion lens for its detection.

This and the following species are included in the genus *Fomes* by Saccardo, and many writers have followed his example. Why this should be done is not clear, for both species are always annual and the tubes are never stratified. The following illustrations will aid in determination: Atkinson, Mushrooms *f. 188*; Bot. Gaz. **46**: *f. 5.*, and Hard, Mushrooms *f. 332.*

61. P. Curtisii Berk. Hooker's Jour. Bot. Kew Gard. Misc. **1**: 101. 1849.

Pileus stipitate, reniform or flabelliform in outline, 3–12 x 3–20 x 0.7–2 cm., coriaceous-corky when fresh, corky when dry, covered with a thin chestnut or reddish varnish that soon begins to disappear, leaving the pileus yellowish or sometimes almost white, glabrous, zonate or concentrically sulcate, the margin rather thick, sometimes truncate; context in two layers, a yellowish or pallid upper layer, rather soft in texture, and a brownish lower layer next to the hymenium, firm or corky in texture, the whole 0.5–1 cm. thick; tubes 0.3–1.2 cm. long, not at all decurrent, the mouths white to brownish, mostly circular, averaging 3–5 to a mm.; stipe always lateral, cylindrical, persistently red-varnished and encrusted, sometimes bluish in color, the context in two layers as in the pileus, 2–10 cm. long, 0.5–3 cm. thick; spores brown, ovoid to elliptic, smooth or appearing minutely echinulate, with a heavy outer wall, 4.6–7.2 x 8.5–11.8 μ.

On and about stumps and trunks of trees. Rare.

This is typically a more southern plant and is rarely found north of the Ohio River. It is distinguished from the preceding species by the yellowish color assumed by the mature pileus, the change in color being due to the disappearance of the reddish varnish. It is sometimes classed as a *Fomes* but is probably never truly perennial. For illustration see Atkinson, Bot. Gaz. **46**: *f. 1–3.*

SPECIES DOUBTFUL OR EXCLUDED

The following species reported by either Morgan or Lea are now believed to have been misdetermined, but the writer does not know to what species the plants should be referred: *P. ovinus* Schaeff. ex Fries; *P. leucomelas* Pers. ex Fries; *P. lentus* Berk.; *P. fragilis* Fries; and *P. badius* Schw.

P. intybaceus Fries reported by Morgan is possibly a form of *P. giganteus*, *P. frondosus*, or a closely related species.

P. phœoxanthus Berk. was originally described from material collected in Ohio by Sullivant. The type specimen is said to be in fragments and the plant has never been collected since Sullivant's time.

FOMES Fries, ex Gill.

Champ. Fr. 682. 1878. Fries; Nov. Symb. 31. 1851.

Plants typically perennial, epixylous, sessile (in our species); pileus corky or more often woody in texture, often becoming rimose, anoderm, or encrusted; context white, reddish, or brownish, soft and punky to hard and woody; tubes as in *Polyporus* except that they are arranged in definite or indefinite layers corresponding to periods of growth of the plant, the mouths circular or angular, never dædaloid or irpiciform; spores white or brown.

The genus *Fomes* includes all of the perennial forms which have the tubes as in the genus *Polyporus*. Each season one layer of tubes is produced and plants of the first season's growth are likely to be referred to the genus *Polyporus*. The key to that genus has been made to include a few such forms, the descriptions of which are always to be sought in the genus *Fomes*. A few species are so constantly annual in duration that they might perhaps better be included in the genus *Polyporus*.

KEY TO THE SPECIES

Context white or only slightly colored (*Species with wood-colored, flesh-colored, or rose-colored context included here*) 1
Context yellowish brown or darker 7
1. Sporophore small, scarcely more than 2 cm. broad; context white 2
1. Sporophore larger, more than 2 cm. broad; context whitish or somewhat colored 3

2. Pileus entirely dark brown or black; plants growing only on the wood of *Alnus* and *Hamamelis* ..... *1. F. scutellatus*
2. Pileus not entirely black, the margin at least remaining white; plant growing on the wood of other deciduous trees, often on structural timber ..... *2. F. ohiensis*
3. Hymenium or context pinkish or reddish 4
3. Hymenium or context whitish or yellowish 5
4. Tubes more than 3 mm. long; plants usually growing on stumps and trunks of *Fraxinus* ..... *6. F. fraxineus*
4. Tubes not more than 3 mm. long; plants usually growing on the wood of coniferous trees ..... *7. F. carneus*
5. Hymenium distinctly stratified, the strata of tubes separated by distinct layers of context; mouths of the tubes angular, usually glistening ..... *4. F. connatus*
5. Hymenium indistinctly stratified or if somewhat distinctly so the layers not separated by distinct layers of context; mouths of the tubes mostly circular, not glistening 6
6. Plant growing on dead wood, usually of coniferous trees; mouths of the tubes small, averaging 3–5 to a mm. ..... *5. F. pinicola*
6. Plant growing only on living *Fraxinus;* mouths of the tubes rather large, averaging 2 to a mm. ..... *3. F. fraxinophilus*
7. Pilei forming a densely imbricate, globose or cylindrical mass ..... *8. F. graveolens*
7. Pilei not forming a densely imbricate, globose or cylindrical mass 8
8. Surface of the pileus not distinctly encrusted 9
8. Surface of the pileus distinctly encrusted 13
9. Context less than 5 mm. thick; sporophore often effused-reflexed or entirely resupinate; growing usually on dead wood ..... *9. F. conchatus*
9. Context more than 5 mm. thick; sporophore generally sessile; often growing on living trees 10
10. Sporophore found only on *Robinia* ..... *11. F. rimosus*
10. Sporophore found on some other host 11
11. Tubes in the older layers distinctly white encrusted or stuffed 12
11. Tubes in the older layers not distinctly white encrusted or stuffed *12. F. Everhartii*
12. Surface of the pileus black, somewhat shining, and rimose; margin rather thin and acute ..... *13. F. igniarius*
12. Surface of the pileus dull brown; margin thick and somewhat obtuse .... *14. F. nigricans*
13. Encrusting layer thin, easily indented; plants annual or sometimes reviving the second season but with the pileus distinct from and coming out below that of the first season ..... *17. F. lobatus*
13. Encrusting layer thick and horny; plants strictly perennial 14
14. Plant growing only on species of *Prunus* ..... *10. F. fulvus*
14. Plant growing on some other host 15
15. Context hard and woody ..... *13. F. igniarius*
15. Context punky 16
16. Mouths of the tubes medium-sized, averaging 3 to a mm.; spores white *15. F. fomentarius*
16. Mouths of the tubes minute, averaging 5 to a mm; spores brown ..... *16. F. applanatus*

1. **F. scutellatus** Schw. ex Cooke, Grevillea **14**: 19. 1885.
Polyporus scutellatus Schw. Trans. Am. Phil. Soc. II. **4**: 157. 1832.

Plants perennial, sessile, often attached by the apex of the pileus; pileus dimidiate or circular, convex, 0.5–1.5 x 0.5–2 x 0.1–0.5 cm., corky when fresh, hard and woody when dry, dark brown or black at least when mature, velvety, azonate or somewhat concentrically sulcate, margin rather thick, acute; context white to wood-colored, corky, not more than 2 mm. thick; tubes 1–2 mm. long, indistinctly stratified, the mouths white to umber, circular or subcircular, averaging 4–5 to a mm.

Chiefly on dead limbs of *Alnus* and *Hamamelis*. Rare.

This species is distinguished from *F. ohiensis* Berk. ex Murrill by the habitat and the black surface of the entire pileus including the margin. Specimens have been received from Mr. Claassen, Cleveland, Ohio.

2. **F. ohiensis** Berk. ex Murrill, Bull. Torr. Bot. Club **30**: 230. 1903.
Trametes ohiensis Berk. Grevillea **1**: 66. 1872.

Plants perennial, sessile, often attached by the vertex of the pileus; pileus dimidiate or shield-shaped, convex to ungulate, 0.5–3 x 0.5–4 x 0.2–1 cm., soft-corky when fresh, hard and woody when dry, at first pure white but becoming cinereous or yellowish and often black at the base but the margin remaining white, finely tomentose to glabrous, often zonate or concentrically sulcate, margin rather thick, acute or obtuse; context white to wood-colored, soft-corky to woody, 1–3 mm. thick; tubes 1–5 mm. long, often arranged in more or less definite rows, indistinctly stratified in two to six layers, the mouths white, circular, averaging 3–5 to a mm., the dissepiments almost as thick as the diameter of the pores.

On dead wood of deciduous trees, especially on structural timber. Common.

By its small size this species is separated from all perennial forms except *F. scutellatus* Schw. ex Cooke. It differs from that species in habitat and in the margin of the pileus always remaining white.

3. **F. fraxinophilus** Peck ex Sacc. Syll. Fung. **6**: 172. 1888.
Polyporus fraxinophilus Peck, Ann. Rept. N. Y. State Mus. **35**: 136. 1882.

Plants perennial, sessile or effused-reflexed, often imbricate; pileus dimidiate, convex to compressed-ungulate, 2–25 x 3.5–40 x 1.5–10 cm., woody, white at first, becoming blackish and often somewhat rimose with age, not encrusted, soon glabrous, concentrically sulcate, margin thick, obtuse or acute; context white to cinnamon wood-color, corky or woody, 0.5–1 cm. or more thick; tubes 2–3 mm. long, indistinctly stratified in many layers, the mouths white to cinereous or yellowish, circular, averaging 2–3 to a mm., the walls thick and entire; spores white, smooth, ellipsoid to ovoid or pyriform, 5–6.3 x 7.3–8 μ.

Growing only on living trunks of *Fraxinus*. Common.

In habitat the species corresponds closely to *F. fraxineus* Bull. ex Cooke, from which it differs in the entire absence of any rosy or reddish colors and in being always perennial. An excellent illustration is given by Hard (Mushrooms *f. 350*).

4. **F. connatus** Weinm. ex Gill. Champ. Fr. **1**: 684. 1878.
Polyporus connatus Weinm. Fl. Ross. 332. 1836. *P. connatus* Fries, Epicr. Syst. Myc. 472. 1838.

Plants perennial, sessile or effused-reflexed, sometimes imbricate; pileus dimidiate, convex, 2–10 x 3–15 x 0.5–4 cm., corky when fresh, somewhat woody when dry, whitish, cinereous, or slightly yellowish, sometimes blackish toward the base, not encrusted, velvety-tomentose to glabrous, usually azonate, margin thick, acute or obtuse; context white or pallid, punky to soft corky, 0.3–1 cm. thick; tubes 1.5–5 mm. long, distinctly stratified, the different strata separated from each other by a thin layer of context, the mouths whitish to yellowish, glistening, angular, averaging 4–5 to a mm., the walls entire to slightly dentate; spores (teste Bresadola) white, globose, 3–4 μ in diameter.

Growing on living deciduous trees, more often at the bases of species of *Acer*, and frequently covered with moss. Common.

The distinguishing characters are the habitat, the layers of context interposed between successive layers of tubes, and the glistening mouths of the tubes. In but one other species of *Fomes* do we find the second character developed and that

is in *F. applanatus* Pers. ex Wallr. That species always grows on old logs and stumps and has a rusty brown context.

Bresadola and Murrill regard *F. populinus* Schum. ex Cooke, to be the same plant as this species. This may be the case but the figure of *F. connatus* in Fries' 'Icones' (*f. 185*) represents our plant much better than the figure of *F. populinus* in 'Flora Danica' (*pl. 1791*). Most of the specimens distributed in exsiccati in both this country and Europe are under the former name and that one is here given preference. A study of the types of both species should show whether they are the same or not, but from the evidence at hand our plants must be referred to *F. connatus*.

5. F. pinicola Sw. ex Cooke, Grevillea **14**: 17. 1885.

Boletus pinicola Sw. Sv. Vet. Akad. Handl. 88. 1810. *Polporus pinicola* Fries, Syst. Myc. **1**: 372. 1821.

Plants perennial, sessile; pileus plane to convex, rarely ungulate, dimidiate, 4–15 x 6–20 x 3–10 cm., woody and rigid, grayish to black, partly or entirely covered with a reddish gluten that forms a crust over the surface, glabrous, sometimes concentrically sulcate, margin thin or thick, often obtuse; context pallid or wood-colored, corky to woody, 0.5–2 cm. thick; tubes 3–5 mm. long, distinctly or indistinctly stratified, the mouths white to umber, circular, averaging 3–5 to a mm., the walls thick and entire.

On dead wood, usually of coniferous trees.

Distinguished from closely related species by the resinous, somewhat sticky, reddish crust found on the pileus. The plant is common wherever coniferous woods are found. Hard (Mushrooms *f. 348*) gives a photograph of it but does not state that he ever collected it in Ohio.

6. F. fraxineus Bull. ex Cooke, Grevillea **14**: 21. 1885.

Boletus fraxineus Bull. Herb. Fr. *pl. 433. f. 2.* 1789. *Polyporus fraxineus* Bull. ex Fries, Syst. Myc. **1**: 374. 1821.

Plants annual or perennial, sessile, sometimes imbricate; pileus dimidiate, 4–10 x 6–15 x 0.6–2 cm., corky when fresh, rigid and woody when dry, light colored, always with reddish or reddish brown stains, or altogether reddish, encrusted with a thin hard crust, minutely velvety to glabrous, more or less zonate, margin thin or thick, acute; context floccose-punky

to corky, whitish or pallid when dry, tinged pinkish or flesh-colored when fresh, 0.2–2 cm. thick; tubes 2–6 mm. long, usually in a single layer but sometimes stratified, mouths whitish, pallid, or flesh-colored, circular or subcircular, averaging 4–6 to a mm., the dissepiments rather thick and entire; spores (teste Murrill) subglobose, smooth, subhyaline, 5–6 x 6–7 μ.

Usually found on living *Fraxinus* but sometimes on other hosts. Rare.

The habitat, the reddish blotches on the pileus, and the pinkish hymenium and context in fresh specimens will identify the plant. But three collections are known from Ohio; one by Morgan, one by W. G. Stover near Columbus, in 1910, and one near Camden, Ohio, by the writer, in 1912. All of these collections are of the annual form.

7. F. carneus Nees ex Cooke, Grevillea **14**: 21. 1885.

Polyporus carneus Nees, Nova Acta Acad. Leop. Carol. **13**: *pl. 3.* 1827.

Plants annual or perennial, sessile; pileus dimidiate, 1.5–5 x 1.5–10 x 0.3–1.5 cm., soft-corky when fresh, firmer when dry, pinkish or rosy, sometimes blackish with age, velvety to glabrous, usually azonate, margin thin and acute; context pinkish or rosy, floccose or punky to soft-corky, 0.2–1 cm. thick; tubes 0.5–3 mm. long, usually in a single layer but sometimes stratified, mouths pinkish or rosy, circular or subcircular, averaging 3–5 to a mm., dissepiments thick and entire.

Usually on wood of coniferous trees. Rare.

The species will be recognized by the uniform pinkish color of the whole plant. The color is well retained on drying. Specimens are in the herbarium of the New York Botanical Garden, collected by James, in Ohio. Authorities disagree as to the identity of *F. carneus* and *F. roseus* Fries ex Cooke.

8. F. graveolens Schw. ex Cooke, Grevillea **13**: 118. 1884.

Boletus graveolens Schw. Syn. Fung. Car. 97. 1822. *Polyporus conglobatus* Berk. Hooker's Lond. Jour. Bot. **4**: 303. 1845.

Plant composed of numerous overlapping pilei arising from a central solid core and forming a more or less globose or cylindrical mass 5–12 cm. in diameter; pilei not more than 2 cm. broad, but connate laterally, corky when fresh, rigid and firm when dry, grayish brown to dull cinnamon-brown, becoming

black in weathered specimens, slightly encrusted, pulverulent to glabrous, azonate or marked with fine grayish zones, margin thick, deflexed and almost concealing the pores; context fulvous to golden brown rust-colored, floccose-fibrous, 1-4 mm. thick; tubes 2–4 mm. long, the mouths grayish, hoary brown, or umber, circular, averaging 3–4 to a mm., the dissepiments thick and entire.

On logs or trunks of deciduous trees, especially *Fagus*, *Quercus*, and *Acer*. Rare.

A characteristic plant that will be recognized at sight. It is commonly known as "sweet knot" from the sweet, powerful odor that it is said to give off. The writer has made four different collections of this rare plant in different stages of growth but has never been able to detect the slightest semblance of a sweet odor. The plant is exceptionally well illustrated by Lloyd (Myc. Notes, Polyp. Issue 3: *f. 367–68;* Syn. Stip. Polyp. *f. 455*), and Hard (Mushrooms *f. 334*). The first and the last of the figures cited are upside down.

9. F. conchatus Pers. ex Gill. Champ. Fr. 1:685. 1878.

Boletus conchatus Pers. Obs. Myc. 1:24. 1796. *Polyporus conchatus* Fries, Syst. Myc. 1:376. 1821.

Plants perennial, sessile, or more often effused-reflexed and frequently entirely resupinate; pileus dimidiate to conchate, 0–7 x 4–12 x 0.2–3.5 cm., woody, grayish brown, yellowish brown or black, rarely encrusted, tomentose at least on the margin, becoming glabrous behind, zonate or concentrically sulcate and sometimes somewhat rimose, margin thin, mostly acute; context yellowish brown to dark brown, woody, 1.5–3 mm. thick; tubes 1–2 mm. long, indistinctly stratified, mouths fulvous to dark brown, usually glistening, circular or subcircular, averaging 4–6 to a mm.

On dead wood, rarely on living trees. Common.

The plant is most frequently found entirely resupinate on old oak logs. Distinctly sessile forms are sometimes found, especially on living trees. The hymenium usually has a silky luster when viewed in changing positions, and on the whole the plant is so characteristic that when once recognized, the collector usually has no trouble with subsequent collections notwithstanding the fact that the species often presents great

differences in size and habit. In Europe the plant is usually known as *F. salicinus* Pers. ex Gill. and it was so reported from Ohio by Morgan. It is entirely different from all other species of *Fomes* except *F. fulvus* Scop. ex Gill. and *F. ribis* Schum. ex Fries in the thin pileus, often conchate in form and with a concave hymenium. Usually the pileus is not more than 1 cm. thick. *F. fulvus* Scop. ex Gill. is distinct in its habitat as is also *F. ribis* Schum. ex Fries.

10. F. fulvus Scop. ex Gill. Champ. Fr. **1**: 687. 1878.

Boletus fulvus Scop. Fl. Carn. **2**:469. 1772. [2nd ed.] *Polyporus fulvus* Fries, Epicr. Syst. Myc. 466. 1838.

Plants perennial, sessile, effused-reflexed or entirely resupinate; pileus dimidiate, convex, 0–4 x 3–8 x 0.5–3 cm., woody, fulvous to ferruginous when young, becoming grayish black or black in age, encrusted, minutely velvety to glabrous, sometimes sulcate, margin rather thick, acute or obtuse; context dark brown, woody, 3–8 mm. thick; tubes 2–4 mm. long, rather distinctly stratified, the mouths circular to slightly angular, grayish brown to tawny, averaging 4–5 to a mm., dissepiments rather thick, entire.

Growing only on wood of species of *Prunus*. Not common.

One should have no trouble in identifying this species if the habitat is taken into consideration as it is the only perennial form that grows on *Prunus*. Morgan reported it under the name of *F. supinus* Schw.

11. F. rimosus Berk. ex Cooke, Grevillea **14**:18. 1885.

Polyporus rimosus Berk. Hooker's Lond. Jour. Bot. **4**:54. 1845. *Pyropolyporus robiniae* Murrill, Bull. Torr. Bot. Club **30**: 114. 1903.

Plants perennial, sessile; pileus dimidiate, convex to ungulate, 3–20 x 6–30 x 1.5–10 cm., woody, at first fulvous, becoming dark brown or black, not encrusted, velvety in young specimens, glabrous and very rimose in old plants, concentrically sulcate, margin thick or thin, obtuse or acute; context fulvous to rusty brown, woody, 0.5–3 cm. thick, zonate; tubes 1–5 mm. long, indistinctly stratified in many layers, the mouths fulvous to rusty brown, circular, averaging 5–6 to a mm., walls rather thick and entire; spores brown, smooth, globose, 4–5μ in diameter.

Growing only on living trunks of *Robinia*. Common.

The type locality for *F. rimosus* is given by Berkeley as the Swan River, Australia, and not Demerara and the Cape of Good Hope, as cited by Saccardo and by Murrill. If the specimens Murrill examined are from the two latter places, it is still possible that our plants belong under *F. rimosus*. Our species also occurs in South Africa as specimens examined from that locality agree well with our plants.

The plant is never found on any other host than the locust tree. This will distinguish it from all of its allies. Its closest relatives appear to be *F. Everhartii* Ellis & Gall. and *F. igniarius* L. ex Gill. The plant is well illustrated by Hard (Mushrooms *f. 347*), and by von Schrenk (Ann. Rept. Mo. Bot. Gard. **12**: *pl. 2*).

12. F. Everhartii Ellis & Gall.[1]

Mucronoporus Everhartii Ellis & Gall. Jour. Myc. **5**:141. 1889.

Plants perennial, sessile or decurrent; pileus dimidiate, convex, rarely ungulate, 2.5–10 x 4–20 x 2–6 cm., woody, entirely fulvous when young but becoming grayish brown or black and rough and rimose with age, velvety when young, glabrous when mature, scarcely encrusted, concentrically sulcate with age, margin thin or thick, acute or obtuse, usually remaining fulvous in color; context fulvous to rusty brown, shining (at least in herbarium specimens), zonate, woody, 1–4 cm. thick; tubes 3–6 mm. long, indistinctly stratified, tubes of the older layers sometimes partly stuffed with mycelium, the mouths concolorous with the context, circular, averaging 4–5 to a mm., the walls rather thin but entire, sometimes glistening; spores distinctly brown, smooth, globose, 4–5.3 μ in diameter.

On living trees, usually of *Quercus*. Not uncommon.

Distinguished from *F. igniarius* L. ex Gill. and *F. nigricans* Fries ex Gill. by the absence of the distinct encrustation or stuffing of the tubes in the old layers, by the more shining context, the somewhat thinner dissepiments, the hyaline spores, and the absence of a distinct crust on the pileus. The two

[1] *F. Everhartii* was originally described under the genus *Mucronoporus* and as far as I have been able to find, no specific statement of transfer to the genus *Fomes* was ever made. At the present time I have not been able to satisfy myself as to who was the first to make (unknowingly, it seems) the new combination, and therefore I do not know to whom credit for the transfer should be given.

species are closely related, however, and without the spores it is sometimes difficult to decide between them. The species differs from *F. fomentarius* L. ex Gill. and *F. applanatus* Pers. ex Wallr. in the unencrusted pileus, the woody context, and the short tubes.

13. F. igniarius L. ex Gill. Champ. Fr. 1:687. 1878.

Boletus igniarius L. Sp. Plant. 1176. 1753. *Polyporus igniarius* Fries, Syst. Myc. 1:375. 1821.

Plants perennial, sessile; pileus dimidiate, convex to somewhat ungulate, 2.5–11 x 4–25 x 1.5–12 cm., woody, grayish black, or entirely black, encrusted, sometimes somewhat rimose in age, glabrous, concentrically sulcate in older plants, margin rather thin, acute, usually grayish in growing specimens; context rusty red or rusty brown, scarcely shining, zonate, woody, 0.5–4 cm. thick; tubes 2–5 mm. long, usually indistinctly stratified, the older layers becoming distinctly whitish encrusted, the mouths circular, grayish brown to dark brown, averaging 4–5 to a mm., the walls thick and entire; spores (teste Romell) hyaline, subglobose, 5–7.5 x 4–7 μ, often 1-guttate.

On trunks of living deciduous trees. Not common.

In no other species is the stuffing or encrusting of the tubes by a whitish substance so evident as in this and the next one. In *F. Everhartii* Ellis & Gall. the tubes appear to be sometimes filled with a whitish mycelium but the character is scarcely evident except on close examination, while in *F. igniarius* and *F. nigricans* Fries ex Gill. in sections through the hymenium the whitish encrustation is plainly visible, and seems to be a distinguishing character. The plant is further to be distinguished from *F. Everhartii* by the hyaline spores, and the thicker dissepiments. The pores are somewhat smaller, but in measuring them the thick dissepiments are included, so that the number per mm. is about the same in the two species. From *F. fomentarius* L. ex Gill. and *F. applanatus* Pers. ex Wallr. the species is separated by the more woody context, the thinner crust, and the much shorter tubes, as well as by the hyaline spores. In *F. igniarius* the pileus is darker in color and is usually much more rimose than in *F. nigricans*. For illustrations see Atkinson, Cornell Univ. Agr. Exp. Sta. Bul. 193: *f. 73–4*.

14. F. nigricans Fries ex Gill. Champ. Fr., Hymen. 1: 685. 1878.

Polyporus nigricans Fries, Syst. Myc. 1:375. 1821.

Plants perennial, sessile; pileus dimidiate, convex to ungulate, distinctly triangular in cross-section, 5–10 x 7–13 x 2–7 cm., woody, dull brown or becoming brownish black, not encrusted, smooth or cracking somewhat in age but scarcely rimose, azonate or with one or two concentric furrows, the margin thick, acute or obtuse, with a broad ferruginous band; context rusty brown, zonate, woody, 0.6–2 cm. thick; tubes 2–7 mm. long, distinctly or indistinctly stratified, becoming distinctly white encrusted or stuffed in the older layers, the mouths dark brown, circular, minute, averaging about 5 to a mm., the walls thick and entire; spores white, subglobose or globose, 6.5 μ in diameter.

On trunks of trees, especially on *Betula*. Not common.

I have one collection of this fungus from W. A. Kellerman. The species has been confused with the preceding one from which it differs in the smoother and differently colored pileus and in being more decidedly triangular in cross-section. The best illustration is that given by Boudier (Ic. Myc. 1: *pl. 155*).

15 F. fomentarius L. ex Gill. Champ. Fr. 1:686. 1878.

Boletus fomentarius L. Sp. Plant. 1176. 1753. *Polyporus fomentarius* Fries, Syst. Myc. 1:374. 1821.

Plants perennial, sessile; pileus dimidiate, convex to strongly ungulate, 3.5–15 x 6–20 x 2–9 cm., hard and woody, grayish to cinereous, brownish, or black, covered with a thick horny crust that appears black and shining when cut, glabrous, smooth, never rimose, zonate or concentrically sulcate, margin thick and obtuse; context fulvous to ferruginous, never shining, punky to soft-corky, zonate, 0.3–3 cm. thick; tubes 0.5–2.5 cm. long, rather distinctly stratified, mouths grayish to cinnamon, averaging 3 to a mm., the walls thick and entire.

On living deciduous trees. Not common.

Distinguished from all of the preceding species by the punky or soft-corky context and the usually longer tubes. Most closely related to *F. applanatus* Pers. ex Wallr. but distinguished from it by the much longer pores and the hyaline spores. For

illustrations see Kellerman, Journ. Myc. **9**: *pl. 3.*, and White, Hymen. Conn. *pl. 35. f. 2.*

16. F. applanatus Pers. ex Wallr. Crypt. Fl. Ger. **2**: 591. 1833.

Boletus applanatus Pers. Obs. Myc. **2**: 2. 1799. *Polyporus applanatus* Fries, Epicr. Syst. Myc. 465. 1838. *P. leucophæus* Mont. Syll. Crypt. 157. 1856.

Plants perennial, sessile; pileus dimidiate, convex or plane, not ungulate, 3–30 x 5–50 x 1.5–7 cm., woody, usually grayish becoming brownish or blackish, glabrous, covered with a thick horny crust, zonate or concentrically sulcate, margin thin or thick, acute or obtuse; context dark ferruginous brown, floccose to soft corky, 0.6–2 cm. or more thick; tubes 0.5–1.5 cm. long, distinctly stratified after the first season, the strata separated by thin layers of context, mouths whitish to umber, darker when bruised, circular, minute, averaging 5–6 to a mm.

On dead wood of deciduous trees or on living trees. Common.

This is our most common species of *Fomes* and may be found in every woodlot, usually on stumps or old logs. It is distinguished from *F. fomentarius* L. ex Gill. by the more applanate pileus and the minute mouths of the tubes. For illustrations see Atkinson, Mushrooms *f. 15.*, White, Hymen. Conn. *pl. 35. f. 1.*, and Atkinson, Cornell Univ. Agr. Exp. Sta. Bul. 193: *f. 82.*

17. F. lobatus Schw. ex Cooke, Grevillea **14**:18. 1885.

Polyporus lobatus Schw. Trans. Am. Phil. Soc. II. **4**:157. 1832. *P. reniformis* Morgan, Journ. Cin. Soc. Nat. Hist. **8**:105. 1885.

Plants annual, frequently reviving for two or three years but the second year's growth distinct from and coming out below that of the first year, sessile or more often appearing substipitate; pileus dimidiate or reniform, plane, depressed, or somewhat convex, never ungulate, 4–12 x 4–15 x 1–3 cm., corky or somewhat flexible when growing, usually umber to yellowish or dark rusty brown, glabrous, covered with a thin, easily indented crust, zonate or concentrically sulcate, margin thin and acute; context dark rusty brown, soft and floccose to punky, 0.3–1 cm. thick; tubes 0.4–1 cm. long, not stratified, mouths circular or subcircular, white, yellowish or umber-

brown, darker when bruised, averaging 4 to a mm., walls rather thin but entire.

On dead wood of deciduous trees. Common.

This species is easily separated from *F. applanatus* Pers. ex Wallr. in that it is not perennial, and in that, if the plant revives the second year, the pileus comes out below that of the first year, and the latter persists as a dead decaying pileus. The second difference is in the character of the encrusting layer of the pileus. In *F. applanatus* Pers. ex Wallr. the crust is hard and horny and one cannot indent it with the thumb nail, while in *F. lobatus* the crust is thin, and often becomes cracked and brittle when old, but is always rather soft and easily indented.

TRAMETES Fries, Gen. Hymen. 11. 1836.

Plants annual or perennial, epixylous, sessile; pileus corky or woody in texture, small or medium sized; context white or brown (never red), descending into and forming the walls of the tubes; tubes typically appearing sunken into the context to unequal depths so that their bases are not in a continuous straight line; mouths circular or angular, never breaking up into teeth and rarely showing a daedaloid tendency.

One species here included in the genus is perennial, all the others are annual. The chief generic distinctions are the unequal depths to which the tubes are immersed in the context, and the homogeneous texture of the context and trama. The first distinction is often not apparent except on very close examination, and at times appears to break down entirely. Consequently, students will meet with some difficulty at times in deciding between the two genera, *Trametes* and *Polyporus*.

KEY TO THE SPECIES

Context white or whitish 1
Context brown or brownish 6
1. Pileus densely hirsute or hispid ..... *7. T. Peckii*
1. Pileus slightly pubescent to glabrous 2
2. Mouths of the tubes minute, averaging 4–6 to a mm. ..... *T. robiniophila*[1]
2. Mouths of the tubes larger, averaging 1–3 to a mm. 3
3. Pileus rather large; context more than 5 mm. thick; plant growing only on *Salix* ..... *3. T. suaveolens*
3. Pileus small, sometimes mostly resupinate; context less than 5 mm. thick; found on some other host 4

[1] For description of this plant see p. 104 under the genus *Polyporus*.

4. Hymenium light brown in color ..... *4. T. malicola*
4. Hymenium white or whitish 5
5. Pileus white or light colored; mouths of the tubes averaging 1–2 to a mm. *1. T. sepium*
5. Pileus cinnamon-brown; mouths of the tubes averaging about 3 to a mm. *2. T. serialis*
6. Sporophore woody, perennial; hymenium bright yellowish brown in color; mouths of the tubes often somewhat daedaloid; growing only on *Pinus* ..... *8. T. Pini*
6. Sporophore coriaceous or corky; hymenium white or dull brown; growing on wood of deciduous trees 7
7. Pileus hirsute or hispid 8
7. Pileus finely tomentose or glabrous 9
8. Mouths of the tubes large, averaging 1 to a mm.; pileus more than 4 mm. thick ..... *7. T. Peckii*
8. Mouths of the tubes medium sized, averaging 2–3 to a mm.; pileus less than 4 mm. thick ..... *6. T. rigida*
9. Context less than 1 mm. thick ..... *5. T. mollis*
9. Context more than 1 mm. thick ..... *4. T. malicola*

1. T. sepium Berk. Hooker's Lond. Jour. Bot. **6**:322. 1847.

Plants annual, sessile or semirespuinate, imbricate or single; pileus dimidiate, 0.7–1 x 0.8–2.5 x 0.2–0.7 cm., flexible when fresh, corky when dry, grayish to pallid or wood-colored, minutely tomentose to glabrous, azonate, margin thin and acute; context white or pallid, tough when fresh, soft-corky when dry, less than 1 mm. thick; tubes 2–5 mm. long, mouths white or pallid, circular or rarely angular or sinuous, large, averaging almost 1 to a mm., the dissepiments thick and always entire; spores (teste Murrill) oblong, smooth, hyaline, 12 x 5 μ.

On fence posts, pickets, and other structural timber or dead wood.

Distinguished from *T. serialis* Fries by the short tubes, the whitish color of the pileus, and by the much larger mouths of the tubes; from *T. rigida* Berk. & Mont. by the lighter colored context, and the larger tube mouths.

2. T. serialis Fries, Hymen. Eur. 585. 1874. [2nd ed.]
Polyporus serialis Fries, Syst. Myc. **1**:370. 1821.

Plants annual, sessile, effused-reflexed, or resupinate; pileus dimidiate, 0–1 x 1–4 x 0.3–0.8 cm., corky when fresh, hard and firm when dry, cinnamon-brown to coffee brown, glabrous, zonate, margin rather thick but acute; context white, fibrous, not more than 1 mm. thick; tubes 2–6 mm. long, the mouths

white or slightly discolored, sometimes slightly glistening, circular to angular, averaging 3 to a mm., the walls firm and entire; spores (teste Bresadola) hyaline, elongate, 7–10 x 3–3.5 μ.

On dead wood. Rare.

The white pores and the internally white tubes contrast strongly with the rich brown color of the pileus. It is distinct from *T. rigida* Berk. & Mont. in the glabrous, thicker pileus. From *T. sepium* Berk. it differs in the much smaller pores and the brown pileus; *T. malicola* has no white color in the tubes and the dissepiments are much thicker.

3. T. suaveolens L. ex Fries, Syst. Myc. 1:366. 1821.

Boletus suaveolens L. Sp. Plant. 1177. 1753.

Plants annual, sessile; pileus dimidiate, 3–9 x 6–14 x 1–3 cm., corky when fresh, firm and rigid when dry, white to grayish or slightly yellowish, finely villous-tomentose to glabrous, azonate, margin thin or thick, acute; context white or pallid, compact-corky to somewhat indurate, 0.5–2 cm. thick; tubes 0.2–1.5 cm. long, the mouths white or cinereous, circular to slightly angular, averaging 1–3 to a mm.

On dead or diseased *Salix*. Rare.

Distinguished from *T. Peckii* Kalchbr. by the prevailing whitish color and the more nearly glabrous pileus.

4. T. malicola Berk. & Curt. Journ. Acad. Nat. Sci. Phil. II. 3:209. 1856.

Plants annual or reviving for two or three seasons, effused-reflexed or entirely resupinate; pileus very narrow, 0–1 x 1–5 x 0.3–0.8 cm., coriaceous and leathery when fresh, corky when dry, avellaneous to cinnamon-brown or wood-colored, azonate, margin thick but acute; context wood-brown or lighter, soft-corky, 2–5 mm. thick; with a distinct pleasant odor when fresh; tubes 2–5 mm. long, sometimes indistinctly stratified in two or three layers, mouths wood-colored to cinnamon-brown, circular to angular or somewhat sinuous, averaging about 2 to a mm., the walls thick and entire; spores white, smooth, oblong, 2.8 –3.5 x 7.5–10 μ.

Growing on dead wood of deciduous trees, especially species of *Acer*. Common.

Entirely distinct from *T. sepium* Berk. in the semiresupinate

habit of growth, the prevailing dull brown color of both hymenium and pileus, and the smaller-mouthed tubes. In this last respect the plant more nearly approaches *T. serialis* Fries and *T. rigida* Berk. & Mont. From the former it is separated by the browner color of the hymenium, the lighter color of the pileus, the internally brown tubes, and the slightly larger and more irregular mouths. From the latter it differs chiefly in the more glabrous and less developed pileus and the longer tubes.

The type specimens of *T. malicola* were collected on the trunk of an apple tree by Schweinitz and referred by him to *P. populinus* Fries. Murrill has placed the name as a doubtful synonym for *P. galactinus* Berk. The writer has not examined the type of *T. malicola*, but our plants bear no resemblance to either *P. populinus* Fries or *P. galactinus* Berk. Our plants were determined by Lloyd and by Bresadola.

5. T. mollis Sommerf. ex Fries, Hymen. Eur. 585. 1874.

Daedalea mollis Sommerf. Suppl. Fl. Lapp. 271. 1826.

Plants annual or rarely reviving, rarely sessile, more often effused-reflexed or entirely resupinate; pileus dimidiate or elongate, 0–2.5 x 1–4 x 0.1–0.5 cm., coriaceous to rigid, umber-brown to almost black, finely tomentose to glabrous, zonate or multizonate, margin thin and acute; context light brown, fibrous, less than 1 mm. thick; tubes 2–3 mm. long, rarely in two or three layers, mouths light brown or grayish, subcircular to somewhat angular, often becoming sinuous or labyrinthiform, averaging 1–3 to a mm.; spores (teste Bresadola) elongate-ellipsoid, smooth, hyaline, 9–11 x 4–4.5 μ.

On dead wood. Rare.

The species differs from *T. rigida* Berk. & Mont. in the distinctly brown and almost glabrous pileus. From *T. serialis* Fries it differs in the light brown context, the much thinner pileus and the usually larger and more irregular pores. The context is much thinner than in *T. malicola* Berk. & Curt. and the general color is decidedly different.

6. T. rigida Berk. & Mont. Ann. Sci. Nat. III. 11: 240. 1849.

Plants annual or rarely reviving, sessile, effused-reflexed or entirely resupinate, sometimes imbricate; pileus dimidiate,

0–3 x 2–6 x 0.1–0.3 cm., coriaceous when fresh, coriaceous or rigid when dry, cinereous to yellowish or slightly brownish, hirsute to hispid, usually zonate, sometimes with multicolored zones, margin very thin and acute; context light umber, fibrous, 0.5–3 mm. thick; tubes not more than 1 mm. long, the mouths white or brownish, circular to somewhat angular, averaging 2–3 to a mm., the walls rather thin but entire.

On dead wood. Not common.

Distinguished from all of its allies in the hirsute or hispid pubescence of the pileus. The pileus is thin and coriaceous and more nearly resembles the thin coriaceous species in *Polyporus*.

7. T. Peckii Kalchbr. Bot. Gaz. **6**: 274. 1881.

Plants annual, sessile or effused-reflexed; pileus dimidiate, 1.5–6 x 2.5–12 x 0.5–2 cm., somewhat coriaceous when fresh, firm and rigid when dry, yellowish brown or reddish brown, densely hirsute or hispid, concentrically sulcate at times, margin thick or thin, acute; context light brown, fibrous, soft and spongy to firm and woody, 1–10 mm. thick; tubes 2–10 mm. long, the mouths dull brown or grayish brown, angular to irregular, averaging about 1 to a mm.; spores (teste Murrill) oblong or slightly curved, smooth, hyaline, 11–13 x 3.5–4 μ.

On dead wood of *Populus*, *Liriodendron*, and *Salix*. September to December. Frequent.

Easily recognized by the densely hirsute or hispid pubescence, the large pores, and the habitat. In Europe the species is known as *T. hispida* Fries.

8. T. Pini Thore ex Fries, Epicr. Syst. Myc. 489. 1838.

Boletus Pini Thore, Essai Chlor. Dep. Land. 487. 1803.

Plants perennial, sessile or effused-reflexed; pileus dimidiate, often ungulate, 3–15 x 5–20 x 1–6 cm., woody, yellowish brown to reddish brown or becoming black, the growing margin hirsute to tomentose, glabrous behind, zonate or concentrically sulcate, margin usually thick and somewhat obtuse; context yellowish brown to rusty brown, corky to woody, not more than 5 mm. thick; tubes 2–7 mm. long, indistinctly stratified, the mouths usually golden brown, subcircular to daedaloid and labyrinthiform; spores (teste Bresadola) hyaline, subglobose, 5–6 x 4–5 μ.

On coniferous wood. Rare.

The bright color of the hymenium usually contrasts strongly with the darker colors of the upper surface. *P. piceinus* Peck (=*Trametes Abietis* Karst.), which by some is regarded as a form of *T. Pini*, has never, to the writer's knowledge, been collected within the state.

SPECIES DOUBTFUL OR EXCLUDED

T. nivosus Berk. was erroneously reported from Ohio by Morgan. It is a tropical and subtropical species.

DAEDALEA Pers. ex. Fries,

Syst. Myc. 1: 331. 1821; Pers. Syn. Fung. 499. 1801.

Plants annual or rarely reviving for two or three years, sessile or effused-reflexed, growing on wood; pileus coriaceous to corky in texture, not encrusted; context white or whitish, fibrous or corky; hymenium typically daedaloid or labyrinthiform, but sometimes poroid, irpiciform or lamellate; spores white.

KEY TO THE SPECIES

Pileus small, thin and coriaceous, hirsute or villous; hymenium at first sinuous and dædaloid but soon breaking up into teeth.....*1. D. unicolor*

Pileus rather large and thick, corky, minutely velvety or glabrous; hymenium poroid, dædaloid, or somewhat lamellate but never breaking up into teeth..... 2

2. Mouths of the tubes less than 1 mm. broad.....*2. D. ambigua*

2. Mouths of the tubes more than 1 mm. broad..... 3

3. Pileus less than 1.5 cm. thick; walls of the tubes thin; plant found abundantly on *Salix*.....*3. D. confragosa*

3. Pileus more than 1.5 cm. thick; walls of the tubes thick; plant growing on *Quercus* and *Castanea*.....*4. D. quercina*

1. D. unicolor Bull. ex Fries, Syst. Myc. 1: 336. 1821.

Boletus unicolor Bull. Herb. Fr. *pl. 408*. 1788.

Plants annual or sometimes the marginal hyphæ reviving and continuing growth the second year, sessile, or effused-reflexed, imbricate; pileus dimidiate to flabelliform, 0.5–5 x 2–8 x 0.2–0.5 cm., coriaceous, white to cinereous or light brown, sometimes green from a covering of algæ, villous or hirsute, zonate or concentrically furrowed, margin thin, acute, sterile below; context white or pallid, fibrous, less than 1 mm. thick; tubes 1–4 mm. long, the mouths white to cinereous or umber, at first

dædaloid and sinuous, but soon breaking up into teeth—though retaining the sinuous character at the margin of the pileus—, averaging about 2 to a mm.

On dead wood. Common.

This plant may at first prove puzzling to the collector, as it was to me when first collected, for the thin, flexible pileus and the usually toothed hymenium indicate a close relationship with the thin coriaceous species of *Polyporus*, or even with *Irpex*. But the pores are decidedly sinuous, at least in young plants. The thin pileus and the hirsute or villous pubescence separate the species from other members of the genus.

2. D. ambigua Berk. Lond. Jour. Bot. 4: 305. 1845.

Trametes lactea Berk. Hooker's Lond. Jour. Bot. 4: 305. 1845.

Plants annual or rarely reviving for two or three years, sessile, sometimes appearing substipitate; pileus dimidiate to reniform, 3–14 x 5–20 x 0.3–1.5 cm., slightly flexible when fresh, corky when dry, pure white to umbrinous, sometimes purplish black at the base, minutely velvety to glabrous, azonate or subzonate on the margin, margin rather thin, acute; context white or pallid, floccose-punky to corky, 0.2–1 cm. thick; tubes 2–4 mm. long, sometimes stratified in two or three layers, mouths whitish or yellowish, circular to sinuous and dædaloid, never lamellate, averaging 2–3 to a mm. in transverse direction, walls rather thick and entire.

On stumps and trunks of deciduous trees. Common.

Distinguished from *D. confragosa* Bolt. ex Fries by the white color, the white context, the smaller pores and the habitat. Hard (Mushrooms *f. 355–56*) gives excellent illustrations of the plant.

3. D. confragosa Bolt. ex Fries, Syst. Myc. 1: 336. 1821.

Boletus confragosus Bolt. Hist. Fung. Suppl. 3: 160. 1791.
Lenzites Cratægi Berk. Hooker's Lond. Jour. Bot. 6: 323. 1847.

Plants annual, sessile; pileus dimidiate, 2–10 x 3–15 x 0.2–1.5 cm., slightly flexible to rigid, grayish or cinereous, rarely slightly brownish, minutely tomentose to glabrous, zonate, margin thin and acute; context whitish, floccose to corky, 0.2–1 cm. thick; tubes 0.1–1 cm. long, mouths whitish to cinereous, sometimes slightly reddish, darker when bruised, subcircular at times but usually sinuous, dædaloid, or labyrinthiform, sometimes becom-

ing lamellate in old plants, 0.5–1.5 mm. broad; spores white, smooth, oblong, mostly curved, 1.5–2 x 6.2–7.5 μ.

On dead wood or on living trees, especially of *Salix*. Common.

This is a very variable species. Sometimes very thin forms are found and such have been considered as species at different times. *Trametes rubescens* Alb. & Schw. ex Fries is a thin form with a reddish hymenium. For illustrations, see Hard, Mushrooms *f. 358.*, White, Hymen. Conn. *pl. 34. f. 2.*, and Moffatt, Higher fungi of the Chicago region *pl. 18.*

4. D. quercina L. ex Fries, Syst. Myc. 1: 333. 1821.

Agaricus quercinus L. Sp. Plant. 1176. 1753.

Plants annual, or sometimes reviving, sessile; pileus dimidiate, convex, 4–12 x 4–15 x 1.5–6 cm., corky, whitish to umbrinous or almost black, glabrous, margin usually thick and obtuse; context whitish, corky, 0.2–1 cm. thick; tubes 1–2 cm. long, the mouths whitish to umber, rarely circular, more often labyrinthiform and elongate or lamellate, 1 mm. or more broad, edges thick and entire.

On *Castanea* and *Quercus*, sometimes on the living trees. Rare.

This species is distinct from all of the others in its habitat, the thickness of the pileus, and the larger sinuous pores. Hard (Mushrooms *f. 357*), and White (Hymen. Conn. *pl. 34. f. 1*) give illustrations of the plant.

LENZITES Fries, Gen. Hymen. 10. 1836.

Pileus coriaceous to corky, dry and floccose in texture. Lamellæ coriaceous, firm, sometimes simple and unequal, sometimes anastomosing behind and forming pores; trama floccose and similar to the pileus, the edge subacute. Dimidiate, sessile, persistent fungi growing on wood and resembling *Dædalea*. (The above description is according to Fries, Epicr. Syst. Myc. 403.)

This genus is intermediate in position between the *Agaricaceæ* and the *Polyporaceæ* and is sometimes included among the white spored genera of the former family.

KEY TO THE SPECIES

Context white ..... *1. L. betulina*
Context brown 1
1. Tubes or interspaces 1 mm. or more broad; lamellæ usually not much anastomosing ..... *3. L. sæpiaria*
1. Tubes or interspaces less than 1 mm. broad; lamellæ freely anastomosing ..... *2. L. vialis*

1. L. betulina L. ex Fries, Epicr. Syst. Myc. 405. 1838.

Agaricus betulinus L. Sp. Plant. 1176. 1753.

Plant annual, sessile, often imbricate; pileus dimidiate, 2–5 x 2–9 x 0.3–1 cm., coriaceous to somewhat corky, prevailing color grayish to brownish, marked with many narrow, multicolored zones, tomentose; margin thin and acute; context white, usually not more than 1 mm. thick; hymenium usually lamellate but sometimes poroid, the lamellæ coriaceous, about 1 mm. apart, 0.3–1 cm. broad, white or whitish; spores globose, smooth, hyaline, 5–6 μ in diameter.

On all kinds of dead wood of deciduous trees. Common.

L. flaccida Fries as reported from Ohio is but a form of this species. The plant is well represented by Hard (Mushrooms *f. 185–86*), Lloyd (Photogr. *pl. 14*), and fairly well in Cooke, Illustrations *pl. 1145*.

2. L. vialis Peck, Ann. Rept. N. Y. State Mus. **26**: 67. 1874.

Dædalea pallido-fulva Berk. Hooker's Lond. Jour. Bot. **6**: 322. 1847.

Pileus annual, sessile, imbricate or single, dimidiate or laterally connate, 1–4 x 2–7 x 0.2–0.8 cm., coriaceous or corky, grayish brown to cinnamon-brown, often darker at the base, subtomentose to glabrous, azonate or subzonate, margin acute; context brown, floccose-fibrous to soft-corky, 1–4 mm. thick; hymenium more or less poroid or labyrinthiform, rarely decidedly lamellate, pores or lamellæ averaging 2–3 to a mm., 1–3 mm. broad, concolorous with the surface of the pileus; spores cylindrical to elliptical, smooth, hyaline, 2.7–4 x 7–8.2 μ.

On dead wood of both deciduous and coniferous trees. Common.

The species was described from Ohio as *Dædalea pallido-fulva* Berk. and so reported by Morgan.

3. L. saepiaria Fries, Epicr. Syst. Myc. 407. 1838.
Dædalea sæpiaria Fr. Obs. Myc. 1: 105. 1815.

Plants annual, sessile, often imbricate; pileus dimidiate or reniform, 1–5 x 2–7 x 0.3–1 cm., coriaceous to corky, bright yellowish red to dark ferruginous, often lighter or discolored with age, strigose-tomentose, zonate, margin thin; context fulvous to ferruginous, floccose to soft-corky, not more than 3 mm. thick; hymenium usually lamellate, the lamellæ about 1 mm. apart, 2–5 mm. broad, rarely anastomosing, fulvous to rusty brown; spores cylindrical, smooth, white, 2.7–4 x 2–10.2 μ.

Always found on dead wood of coniferous trees. Frequent.

Easily distinguished from the preceding species by the deeper color throughout and by the more distant lamellæ that rarely anastomose.

CYCLOMYCES Kunz. & Fries, Linnæa **5**: 512. 1830.

Plants annual, terrestrial and stipitate in our species, coriaceous, fuscous or cinnamon-colored; context brownish, sometimes rusty brown, floccose to fibrous; hymenium poroid at first but soon breaking up into concentric lamellæ.

The genus is distinct from all others in the concentric arrangement of the lamellæ.

1. C. Greenei Berk. Hooker's Lond. Jour. Bot. 4: 306. 1845.

Pileus stipitate, circular in outline, usually depressed on top, 2.5–9 cm. broad, 0.5–2 cm. thick, coriaceous when fresh, rigid when dry, yellowish brown to rusty or purplish brown, tomentose at first but becoming glabrous, more or less zonate, margin thin and acute; context fulvous to cinnamon-brown, soft floccose to fibrous or somewhat friable, thin at the margin, thicker next the stipe; tubes 5–8 mm. long, soon breaking up to form brownish concentric lamellæ; stipe central or subcentral, expanding above into the pileus, velvety, somewhat spongy, 2–7 cm. long, 0.7–2 cm. thick, fulvous to rusty brown in color.

On the ground in woods. Rare.

The species was reported from Ohio by Hard but I think has not otherwise been collected. For illustration see Hard, Mushrooms *f. 360–61*.

FAVOLUS Fries, Elench. Fung. 1: 44. 1828.

Plants annual, epixylous, more or less stipitate; pileus fleshy-tough when fresh, small or medium sized; context white, thin; tubes in a single layer, the mouths angular, usually hexagonal, often radiating outward from the stipe and somewhat longer in the radial direction; spores white.

In our species the stipe is much reduced and is usually lateral or at least eccentric. The genus is separated from *Polyporus* by the large favoloid pores, although some stipitate species of *Polyporus* closely approach in pore form the condition ascribed to this family.

KEY TO THE SPECIES

Plants about 2 cm. long and broad; hymenium more or less waxy or gelatinous.....*1. F. rhipidium*
Plants larger than above; hymenium not gelatinous or waxy.....*2. F. canadensis*

1. **F. rhipidium** Berk. Hooker's Lond. Jour. Bot. **6**: 319. 1847.

Plants stipitate; pileus reniform, cæspitose-imbricate, 2 cm. long and broad, coriaceous, alutaceous to white, the cuticle breaking up into minute furfuraceous squamules, concentrically sulcate; context whitish, thin; tubes short, less than 2 mm. long, more or less waxy and gelatinous, the mouths white, angular to elongate, denticulate, averaging 2–3 to a mm.; stipe lateral, pruinose, 6–7 mm. long.

On dead wood. Rare.

The above description is adapted from the original. The species was originally described from Ohio from specimens collected by Lea. Morgan also probably collected it, but otherwise it is not known from the state. In habit and color it resembles *Panus stypticus*.

2. **F. canadensis** Klotzsch, Linnæa **7**: 197. 1832.

F. ohiensis Berk. & Mont. Syll. Crypt. 171. 1856. *F. striatulus* Ellis & Ev. Am. Nat. **31**: 339. 1856.

Plants stipitate, the stipe often reduced to a lateral tubercle; pileus dimidiate to reniform, 1–4 x 1–8 x 0.1–0.7 cm., fleshy-tough when fresh, rigid when dry, at first reddish brown due to the presence of innate fibrils of that color, later becoming glabrous and fading to cream color or pure white, azonate,

margin thin and acute, often involute, especially on drying; context white or whitish, fleshy-tough, becoming firmer on drying, 0.5–2 mm. thick; tubes 1–5 mm. long, the mouths whitish to yellowish, distinctly angular, usually rhomboid or hexagonal, often radiating outward from the stem and longer in the radial direction, very variable in size, 0.5–3 mm. long and averaging 1–3 to a mm. in transverse direction; stipe lateral or rarely subcentral, often rudimentary, not more than 1 cm. long, 1.5–7 mm. thick.

On dead branches of deciduous trees, especially *Hicoria*. Common.

F. striatulus Ellis & Ev. is supposed to differ from *F. canadensis* in having a pileus white in color from the first, and in the smaller pores. In Ohio both of these forms are found and the writer has come to the conclusion that *F. striatulus* is to be regarded as only a form of this rather polymorphic species, for the following reasons: First, specimens of *F. canadensis* frequently become whitish in color quite early in development; second, the small pores said to be characteristic of *F. striatulus* are also frequently found in specimens with the reddish brown pileus. In attempting to separate the plants into two species one finds reddish brown specimens with either large or small pores, and white specimens with either large or small pores. The species is illustrated in Hard, Mushrooms *f. 359*.

GLOEOPORUS Mont. Hist. Cuba 385. 1838.

Plants annual, sessile or effused-reflexed; pileus small, thin and coriaceous; context fibrous, thin, usually white; tubes short, more or less gelatinous or waxy and in our species separating from the context in a thin, elastic layer when fresh or when moistened. The genus is distinct from all others in the gelatinous and at the same time separable hymenium. One species only is found in our flora.

1. **G. conchoides** Mont. Hist. Cuba *pl. 15. f. 1.* 1838.

Sessile or effused-reflexed; pileus dimidiate or conchate, 0.5–3 x 1–4 x 0.1–0.5 cm., coriaceous when fresh, rigid when dry, white or cream-colored, velvety to glabrous, azonate, margin thin, acute, with a narrow sterile band below; context white, soft-fibrous, 1–4 mm. thick; tubes less than 1 mm. long,

gelatinous or waxy and separating from the context in a thin elastic layer when fresh or when moistened, the mouths flesh-colored to reddish purple or purplish black, circular, minute, averaging 5–6 to a mm.

On dead wood of deciduous trees. Common.

The waxy separating hymenium, reddish purple in color, will serve to distinguish this species. The plant has been known as *Polyporus dichrous* Fries.

MERULIUS Haller ex Fries,
Syst. Myc. 1: 326. 1821; Haller, Hist. Stip. Helv. 3: 150. 1768.

Hymenophore formed from a mycelial mucedinous context and giving rise to shallow irregular pores formed by the intersection of obtuse folds of the hymenium; resupinate or pileate, more or less waxy in texture. Growing on rotting wood.

This genus is a very natural one and forms a transition stage from the *Polyporaceæ* to the *Thelephoraceæ* through the genus *Phlebia* of the *Hydnaceæ*. No special study of the genus has been made and only the two common species are included here, although several others have been reported from the state.

KEY TO THE SPECIES

Pileus always present, distinctly pinkish red when fresh ..... *1. M. rubellus*
Pileus when present whitish or somewhat flesh-colored but not distinctly pinkish red ..... *2. M. tremellosus*

1. M. rubellus Peck, Bot. Gaz. 7: 44. 1882.

Pileus sessile or effused-reflexed, dimidiate, often imbricate, 3–5 x 5–7.5 x 0.2–0.5 cm., coriaceous-cartilaginous, scarcely waxy or gelatinous, deep pinkish red, often fading with age, finely tomentose, azonate, margin thin, acute; context white or light colored, tough when fresh, soft when dry, 1–4 mm. thick; tubes short, less than 1 mm. long, formed by anastomosing veins, averaging 1–2 to a mm., cream-colored or whitish; spores (teste Peck) minute, elliptical, hyaline 4–5 x 2.5–3 μ.

On dead wood of deciduous trees. Common.

This plant is distinguished from the next one by the firmer consistency and the color, although the color of the pileus often fades in mature plants. Hard (Mushrooms *f. 353*) gives a good illustration of the plant.

2. M. tremellosus Schrad. ex Fries, Syst. Myc. 1: 327. 1821.

M. tremellosus Schrad. Spic. Fl. Ger. 139. 1794.

Sessile, effused-reflexed, or entirely resupinate; pileus dimidiate, 0–5 x 3–8 x 0.1–0.3 cm., fleshy or gelatinous-waxy, white or whitish, tomentose, azonate, margin thin and acute; context whitish, soft, 1–2 mm. thick; tubes very short, formed by anastomosing ridges or veins, averaging 1–2 to a mm., whitish or somewhat flesh-colored, in resupinate forms with a wide, thin, sterile border.

On old logs in woods. Common.

Quite often the plant is entirely resupinate and probably always so in young stages. The form of the hymenium is exceptionally well shown in Atkinson, Mushrooms *f. 191–92.*

Besides the above species, *M. lacrymans* Jacq. ex Fries has been included in practically every list of fungi reported from the states east of the Mississippi River, but its frequency of occurrence is probably in inverse ratio to the number of times reported. At any rate it is to be considered as a rare fungus in this country. I have never met with specimens in Ohio that I could so refer.

IRPEX Fries, Elench. Fung. 1: 142. 1828.

Hymenium inferior, dentate–lacerate from the first. Teeth concrete with the pileus, firm, subcoriaceous, acute, reticulately disposed or arranged in rows, in sessile forms connected at the base and gill-like, or favoloid in resupinate forms. Basidia 4-spored. Woody, subsessile or resupinate fungi allied to *Lenzites* and *Dædalea.* (Adapted from Fries, Hymen. Eur. 619.)

This genus is sometimes included in the *Hydnaceæ* but in at least one of the three species here described the hymenium is not toothed from the first, but is decidedly poroid and shows very close relationships to certain species of the thin pileate members of the genus *Polyporus*, e. g., *P. biformis, P. prolificans* etc., in which the hymenium soon becomes broken up into teeth. For this reason and because the plants are very common in our woods the three following species are described and most of the collections usually obtained will be found to answer to one of these descriptions.

KEY TO THE SPECIES

Context white or whitish 1
Context brown or brownish 2

1. Context less than 2 mm. thick; tubes or teeth less than 5 mm. long; pileus villous ..... *1. I. tulipifera*
1. Context more than 2 mm. thick; tubes or teeth more than 5 mm. long ..*2. I. mollis*
2. Hymenium cinnamon-brown ..... *3. I. cinnamomeus*
2. Hymenium grayish green to olivaceous ..... *4. I. farinaceus*

1. **I. tulipifera** Schw. ex Fries, Epicr. Syst. Myc. 523. 1838.
Boletus tulipifera Schw. Syn. Fung. Car. 99. 1822.

Plants sessile, effused-reflexed, or entirely resupinate; pileus dimidiate to elongate in outline, 0–1 x 1–3 x 0.1–0.6 cm., coriaceous, white or whitish, villous, zonate, margin thin and acute; context white, fibrous, 0.5–2 mm. thick; tubes 1–5 mm. long, the mouths light colored, averaging 2 to a mm., soon breaking up into compressed teeth that are connected at the base, and often with a concentric arrangement.

On dead wood of deciduous trees. Common.

From *I. cinnamomeus* Fries, and *I. farinaceus* Fries this plant is separated by the white or whitish color, and from *I. mollis* Berk. & Curt. by the much thinner pileus and the shorter tubes or teeth.

2. **I. mollis** Berk. & Curt. Jour. Bot. & Kew Misc. 1: 236. 1849.

Pileus sessile or effused-reflexed, dimidiate, 2–5 x 5–10 x 1–3 cm., coriaceous, white or whitish, minutely tomentose to glabrous, azonate, margin thin and acute; context white, 2–6 mm. thick, fibrous; hymenium usually irpiciform, the teeth white, coriaceous, 0.5–1.5 cm. long, compressed, united at the base.

On dead wood of deciduous trees.

This plant was reported from the Miami valley by Morgan. I have not collected it in Ohio. It is much thicker than *I. tulipifera* Schw. ex Fries, and the teeth are much longer.

3. **I. cinnamomeus** Fries, Epicr. Syst. Myc. 524. 1838.

Pileus none, fungus usually entirely resupinate, coriaceous in texture, 2–5 mm. thick, entirely cinnamon-brown; context brown, not more than 1 mm. thick, fibrous; tubes or teeth 1–5 mm. long, becoming toothed at a very early stage, cinnamon-brown in color, more or less flattened, connected at the base.

On dead wood, especially of species of *Acer*. Rather common.

Distinguished from the other species here listed by the uniform brown color.

4. I. farinaceus Fries, Linnæa **5**: 523. 1830.

Pileus sessile, effused-reflexed, or resupinate, dimidiate, 0–0.5 x 1–3 x 0.1–0.3 cm., coriaceous, deep brown, finely tomentose, zonate, margin thin and acute; context dark brown, fibrous, less than 1 mm. thick; tubes 0.5–1.5 mm. long, mouths usually grayish green or yellowish green, averaging 2–3 to a mm., soon breaking up into teeth.

On dead wood of deciduous trees. Not common.

Sometimes the fungus is entirely resupinate and then it usually has a narrow brown margin. It is distinct from all of the other species in having a greenish hymenium.

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Names in italics are synonyms, rejected species, etc.

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A CONTRIBUTION TO OUR KNOWLEDGE OF THE RELATION OF CERTAIN SPECIES OF GRASS-GREEN ALGÆ TO ELEMENTARY NITROGEN

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A general survey of the literature pertaining to the relation of algæ to free atmospheric nitrogen reveals the fact that comparatively few forms have been experimented with under conditions which render the conclusions reached free from objection. The principal fault which may be found with most of the work done is that the experiments were carried out with impure cultures. Representatives from not more than four or five genera of green algæ have thus far been studied in pure culture, and while the general conclusion reached is that these forms are unable to fix free atmospheric nitrogen either in the presence or in the absence of combined nitrogen and energy-furnishing materials, it is by no means certain that forms do not exist which, under one or all of these conditions, are able to utilize elementary nitrogen. This thought is especially justified when the small number of free-nitrogen-fixing species among the bacteria is considered. In the present investigation, therefore, an attempt has been made to extend the observations over a greater variety of forms in pure culture,—understanding by the latter a single species of alga free from all other organisms.

HISTORICAL

As early as 1854 Laurent (20, 21), and Morren (24) occupied themselves indirectly with the relation of algæ to free atmospheric nitrogen. Morren was led to the conclusion that the sudden death of cultures of infusoria and algæ was due to the insufficient quantity of combined nitrogen furnished when the number of organisms became considerable. The nitrogen requirement, he found, could be satisfied by ammonium carbonate, organic nitrogenous compounds (decaying insects), and other nitrogenous substances in the water; but in no case did he find that free nitrogen from the atmosphere could serve as the source of nitrogen. While it is difficult to say with what organisms Morren worked, it is altogether probable that members of the *Volvocaceæ* were present among his "green," "brown," and "red infusoria."

No additional contribution to the subject, so far as the author is aware, was made until the appearance of Frank's paper (9) in 1888. In his investigation of the question of a possible fixation of free atmospheric nitrogen in natural soil without the instrumentality of cultivated plants, Frank exposed samples of unsterilized soil, poor in organic matter, in containers under a glass roof, watering them only with distilled water. During the 134 days that the experiment was continued, no phanerogams appeared, but in all cases the surfaces of the soil samples became covered with a thin, crustlike, greenish layer composed of "zwei spangrüne Oscillariaformen, die eine dick–, die andere sehr dünnfädig; ferner grünes Chlorococcum humicola, vielleicht auch Pleurococcus, sowie Vorkeimfäden von Moosen, also kryptogame Gewächse . . . Diatomaceen waren nicht zu finden." Analysis showed an undoubted increase in total nitrogen in the experiments. No increase in the nitrate content was observed,—the additional nitrogen being wholly in the form of organic nitrogenous compounds. These facts led the author to the conclusion that the abundance of algal cells, which are rich in protoplasm and therefore in organic nitrogen, accounts for the presence of the increased nitrogen in an organic form. That the appearance of the nitrogen in an organic form (algal substance) does not represent the primary fixation of free nitro-

gen and that the latter depends on an inorganic process, the inorganic compounds thus produced being subsequently assimilated by the algæ, is not rendered probable by later experiments. In these, Frank exposed samples of soil, kept free from vegetation, for long periods of time and at various temperatures. Plant growth was prevented by leaching the samples daily with hot water. In this manner any traces of nitrogen compounds formed were also obtained. Only at high temperatures—too high for plant growth—did he find a slight increase in total nitrogen and therefore believes that this process is of no importance under conditions which admit of plant growth. From these observations Frank concludes that the algæ themselves are the immediate agents in the fixation of free atmospheric nitrogen and inclines to extend this faculty to green plants in general.

In the same year, Gautier and Drouin (11) ascribed an entirely different function to soil algæ. Samples of artificial soils, free from organic material and containing only ammoniacal nitrogen, were exposed in a sheltered position for a considerable period of time. During the progress of the experiments the soil became more or less covered with a layer of green algæ (*Pleurococcus vulgaris*, *Protococcus viridis*, etc.). Analysis showed, in every case, a loss in total nitrogen, an even greater loss in ammoniacal nitrogen, and an intermediate gain in organic nitrogen. The authors assumed that the nitrogen lost was in the form of ammonia and that the amount of nitrogen appearing in the organic form was that part of the escaping ammoniacal nitrogen which, in bathing, so to speak, the algal cells on the surface, was absorbed, and subsequently built into organic nitrogen compounds. In support of this hypothesis the authors state that in proportion to the intensity of the algal growth loss in total nitrogen was diminished, and the amount of ammoniacal nitrogen converted into organic nitrogen increased. Gautier and Drouin thus looked upon the algæ as fixers of gaseous ammonia, which the soil tends to give off constantly, rather than as direct agents in the fixation of free atmospheric nitrogen.

In 1889, Frank (10) made the fixation of elementary nitrogen by soil-inhabiting algæ the subject of a special investigation. Four flasks containing sand moistened with distilled water and

plugged with cotton were treated as follows: Two were at once placed in the light; the third was covered with black paper and without further treatment placed with the first two; the fourth was exposed for six hours to a temperature of 100°C. and then placed with the rest. In the first two, rich algal growths developed, composed of two species of *Oscillatoria,* a blue-green "*Nostoc*–Form," a yellowish green "*Nostoc*–Form," a yellowish to pure green *Microcystis*, and a *Glœocapsa.* In the third and fourth flasks no growth of any kind developed. Analyses demonstrated that the total nitrogen content in the first two flasks had been doubled, whereas that in the latter two had suffered a distinct loss. The experiments were repeated with unsterilized soil, all air gaining access to the flasks being first passed through sulphuric acid to remove any ammonia present. The same characteristic algal flora developed and analysis again showed a decided increase in total nitrogen. On the basis of these experiments, Frank makes the generalization that the soil, as such, is unable to fix free atmospheric nitrogen, and that when the process does take place, it is effected by means of the vegetation of low algæ which develop in the soil, and which possess the ability of assimilating free gaseous nitrogen into vegetable, nitrogen-containing compounds. He goes still farther and states that the fact that low algæ utilize free nitrogen makes it more and more probable that the assimilation of elementary nitrogen is a faculty appertaining to the entire plant world provided with chlorophyll, and that, since the simple algal cell is endowed with this faculty, the thought is justified that the assimilation of free atmospheric nitrogen is as absolute and fundamental a process of the entire plant kingdom as is the assimilation of carbon dioxide.

Prantl (27), in cultivating fern prothallia in solutions with and without combined nitrogen, observed that whereas an abundant algal vegetation appeared in the former, only an *Anabœna*, or a *Nostoc*, grew in the latter. When placed in nitrogen-free media, the blue-green alga always grew abundantly. From this observation, and without analytical data, Prantl assumes that free-nitrogen assimilation had taken place, either a direct one by the alga, or an indirect one in which the alga assimilated the ammonium nitrite which, according to the theory of Schoenbein,

is formed in the vaporization of water. Of interest are the observations by the same author on the unicellular grass-green algæ, which he was unable to cultivate in solutions free from combined nitrogen. To these, therefore, he assigned the power of elementary-nitrogen fixation in a much smaller degree than to *Nostoc*.

Frank's conclusions were confirmed by the work of Schloesing and Laurent (31). These investigators supplemented the usual indirect method of analyzing the soil and harvest, with the direct method of determining at the beginning and at the end of the experiment the composition of the atmosphere in which the plants had been growing. To 2000 or 2500-gram quantities of a poor sandy soil 2.5 grams of limestone, 5 grams of a mixture of several rich soils, and a certain volume of a mineral nutrient solution containing, in some cases, a little potassium nitrate were added, and the whole placed in large flasks. In some, seeds of Jerusalem artichoke, oats, peas, and tobacco were planted; others, to be used as checks, remained unplanted. To each flask were added 5 cc. of a liquid obtained by diluting 5 grams of rich soil with 20 cc. of water. After fourteen weeks, during which time the seeds germinated and produced plants, the direct analytical method, confirmed by the results obtained by the indirect method, showed, except in two checks, an absorption of free atmospheric nitrogen. But the surfaces of the soils, during the progress of the experiments, became covered with green, cryptogamic plants, among which were mosses (*Bryum*, *Leptobryum*), and algæ (*Conferva*, *Oscillatoria*, *Nitzschia*). This fact led the authors to repeat the first series of experiments, in every case suppressing the growth of chlorophyllous cryptogams by covering the soils with a thin layer of dry, calcined, quartz sand. No trace of algæ or mosses appeared, and, except in the case of the peas, no absorption of free atmospheric nitrogen was observed. This fact, together with the evident fixation of nitrogen in the checks of the first series (in which an abundant chlorophyllous cryptogamic vegetation but no phanerogamic vegetation developed), and the absence of fixation in those checks in which little or no algal growth developed, led Schloesing and Laurent to conclude that there are some "inferior green plants" which are able to utilize free atmos-

pheric nitrogen. In the same year, Gautier and Drouin (12) reasserted their former conclusion as to the rôle of algæ in nitrogen fixation, holding that the methods of those who adhere to the opinion that algæ fix free nitrogen are too faulty to make conclusions drawn from them convincing.

In the work reported by Schloesing and Laurent in 1892 (32, 33) an attempt was made to reduce the complexity of the algal cultures by introducing into a single experiment only one or at most a few species of the algæ. All cultures were made on 600-gram quantities of either a subsoil or quartz sand to which was added (except in the two checks) a small quantity of an infusion prepared from soils. The cultures were allowed to develop for from three to six months, and, as in the previous experiments of these authors, analyses were made both of the contained atmosphere and of the soil and algal growth. The chlorophyllous plants which appeared in the various cultures are described as follows: I and II—essentially a mixture of *Nostoc punctiforme* Hariot and *Nostoc minutum* Desmazières, with a few colonies of *Cylindrospermum majus* Kuetz.; III—almost a pure culture of *Nostoc punctiforme;* IV—*Nostoc punctiforme* (less pure than in III), one colony of *Phormidium papyraceum,* and a small quantity of *Nostoc minutum;* V—two mosses—*Brachythecium rutabulum* and *Barbula muralis;* VI—an almost pure culture of an *Oscillarieæ* and *Microcoleus vaginatus,* with traces of *Tetraspora, Protococcus, Stichococcus, Ulothrix,* and *Lyngbya;* VII and VIII—checks with no growths, or at most a few small patches of *Phormidium autumnale* Gomont and *Nostoc punctiforme.* Both analytical methods showed abundant nitrogen fixation in the first four cultures but not an appreciable one in the fifth,—a fact which the authors explain on the basis of specific differences in plants in their ability to fix free atmospheric nitrogen. The checks showed no appreciable fixation. Separate analyses were made of the top-soil layers, containing the algal growths, and the deeper layers, the increased nitrogen being found in the algal stratum,—a fact which the authors consider important in proving that the algæ were responsible for the free-nitrogen fixation. In conclusion, Schloesing and Laurent admit the possibility that the bacteria present in the cultures had something to do with the fixation of free

nitrogen, and state that it is not possible to affirm with certainty that the algæ, free from other organisms, are able to effect fixation. Having observed, however, but few bacteria in the cultures they conclude that the algæ after all are the active agents in the fixation of elementary nitrogen.

Similar results were obtained by Koch and Kossowitsch (17). Sixty grams of washed, calcined sand were placed in large Erlenmeyer flasks and moistened with a mineral nutrient solution free from combined nitrogen. Since previous experiments had shown that algæ do not grow on sand free from combined nitrogen, 0.04 gram of calcium nitrate dissolved in 50 cc. of water were added to each flask. After inoculation with a suspension of algal cells obtained from heaps of lime, a continuous slow stream of air, washed in sulphuric acid, was passed through all the flasks. Three cultures were placed in a north window, three in the dark (to determine whether the bacteria contained in the cultures fixed free nitrogen), and the remainder were used in determining the initial total nitrogen. After fifteen weeks, during which time a rich algal vegetation[1] developed on all cultures exposed to the light, the contents of the flasks were analyzed *in toto*. Those exposed to the light showed an undoubted increase in total nitrogen, whereas those in the dark showed a slight loss in each case. Of particular interest was one culture which was brought into the light after it had remained in the dark for a considerable length of time. After the removal, a moderate growth of algæ appeared, and analysis showed a slight gain in total nitrogen, which, however, was less than that found in the cultures which had been exposed to the light during the entire period. In agreement with the earlier workers, these authors ascribed to algæ the faculty of free-nitrogen fixation, and emphasized the observation that the extent of this fixation was directly proportional to the intensity of the algal development. Petermann (26) reached a similar conclusion on the basis of experiments conducted on sterilized and unsterilized soils, which were respectively inoculated and uninoculated with algæ. The former in each case showed a distinct gain in nitrogen, whereas the latter showed either no increase or a slight loss.

[1] The authors failed to state what algæ developed, merely mentioning the presence of green and blue-green forms.

Incidental to his work on the respiratory quotient in algæ, Schloesing (30) reported that in a culture containing principally *Protococcus vulgaris* Ag., and smaller quantities of *Chlorococcum infusionum* Menegh., *Ulothrix subtilis* Kütz., and *Scenedesmus quadricauda* Bréb., there was at the end of two months no diminution of nitrogen in the supernatant atmosphere. This fact led the author to place these algæ among those forms which do not fix free atmospheric nitrogen.

As will have been observed, the work reported upon in the contributions cited was done with impure cultures. While in some cases but a single species was used, bacteria were present in all cases. Although in many instances this is not expressly stated, the author's experience convinces him that the technique employed by these earlier workers made the contamination of their cultures with bacteria very probable. It is evident, therefore, that in the work done thus far it is impossible to state with certainty whether the results obtained are due to the activity of the algæ, or to the bacteria, or to both.

The first work done on the fixation of free nitrogen by algæ in which pure cultures were used was that of Kossowitsch (18), in 1894. The only form isolated in pure culture by this investigator was one which he states resembled both *Cystococcus* (Nägeli) and *Chlorella vulgaris* Bey. He leaves its identity uncertain but designates it, for convenience, *Cystococcus*. Preliminary experiments with impure cultures of this alga had demonstrated that asparagin and ammonium tartrate could not serve as the source of nitrogen and that growth took place only when nitrates were supplied. In the experiments with pure cultures, flasks containing 70 grams of clean sand moistened with a mineral nutrient solution containing a known amount of calcium nitrate were inoculated with a carefully tested pure culture of *Cystococcus* and allowed to remain four months. To a number of the cultures dextrose was added, and to others, in addition to this sugar, pea-tubercle bacteria. At the conclusion of the experiments the cultures were carefully tested for purity. Analysis in every case showed an absence of free-nitrogen fixation, and demonstrated clearly for the first time that an alga, *Cystococcus*, under the conditions realized in the experiment, did not fix free atmospheric nitrogen. That the

same holds true for this alga in nature seemed probable to Kossowitsch, who found that it grew vigorously only so long as a nitrate was present. He further observed that after growth had ceased in any culture, it was promptly resumed upon the addition of a nitrate solution, but not when the nitrogen-free nutrient solution was added. Similar cultures were started in which the inoculation material was either a mixture of algæ and bacteria derived from soil or lime, or a mixture of soil bacteria with a pure culture of *Cystococcus*. In each case the cultures were set up with and without dextrose. Table I gives the results of these experiments.

TABLE I

RESULTS OF KOSSOWITSCH'S EXPERIMENTS WITH PURE AND MIXED CULTURES

+ or − Sugar	Content of cultures	Mg. of N in cultures	
		Initial	Final
−	*Cystococcus* (pure culture)	2.6	2.7
+		2.6	2.7
−	*Cystococcus*, *Phormidium*, soil bacteria, moulds	2.6	7.1
+		2.6	9.5
−	Pure *Cystococcus* culture and bacteria	2.6	3.1
+		2.6	8.1
−	*Stichococcus* and bacteria	2.6	2.3
+		2.6	2.7
−	*Nostoc*, large round alga, *Scenedesmus*, soil bacteria	2.6	?
+		2.6	19.1
−	*Nostoc*, a *Cylindrospermum* (small form), soil bacteria	2.6	8.8
+		2.6	25.4

Cystococcus, in pure culture, was again unable to fix free gaseous nitrogen, and the same conclusion is reached by Kossowitsch for *Stichococcus*, which even in the presence of a mixture of bacteria failed to fix elementary nitrogen. Of especial interest are the cultures of pure *Cystococcus* with bacteria, as in these the fixation is ascribable only to the bacteria. Which of the organisms in the remaining cultures are responsible for

the marked fixation of free atmospheric nitrogen it is impossible to say, the author states. However, from his own results, and those of previous investigators, that the presence of algæ exercises a favorable effect on the process of free-nitrogen fixation, and, further, that the algæ thus far studied in pure culture do not possess this faculty of fixation, Kossowitsch concludes that the algæ play an indirect rôle. He believes they do this by furnishing, through their photosynthetic activity, carbohydrates to the nitrogen-assimilating bacteria. He would look upon the algæ as occupying the same position with reference to free-living, nitrogen-fixing bacteria as the legumes do with reference to the nodule organisms.

Stocklasa (35), while not making his conclusion very clear, leads one to believe that he considers certain algæ (which he fails to enumerate) capable of fixing free atmospheric nitrogen. Unfortunately, all of Stocklasa's experiments were carried out with impure cultures. Molisch (23), in conducting experiments with algæ relative to the necessary nutrient elements, attempted to cultivate *Microthamnion Kützingianum* Näg., *Stichococcus bacillaris* Näg., *S. major* Rbh., *Ulothrix subtilis* (?) Kütz., and *Protococcus sp.*—all in impure culture—on a nitrogen-free mineral nutrient solution. In every case the algæ failed to grow, and Molisch was led to the conclusion that algæ require combined nitrogen for their development. Although no experiments in which combined nitrogen was furnished to the algæ were conducted, the author nevertheless makes the statement, based principally on the work of Kossowitsch just reviewed, that algæ are not able to fix free atmospheric nitrogen.

In the next year Bouilhac (4) reported that he had succeeded in isolating in pure culture *Schizothrix lardacea, Ulothrix flaccida,* and *Nostoc punctiforme.* Unfortunately, this author does not give a detailed account of his isolation methods. Six flasks containing a mineral nutrient solution free from combined nitrogen were inoculated with each alga, and to three of each a drop of soil suspension was added. No growth whatever developed in any of the *Schizothrix* and *Ulothrix* cultures, nor in the *Nostoc* cultures to which the suspension had not been added. But in those cultures of the latter to which a drop of soil suspension had been added, a splendid growth appeared and in each

culture analysis showed a nitrogen fixation of from 11 to 23 milligrams. From a second series (in which the cultural solution contained per liter 0.1 gram arsenic acid in the form of potassium arsenate) a similar result was obtained, with fixation of nitrogen of from 5 to 60 milligrams. The presence of *Ulothrix* or *Pleurococcus* in addition to the *Nostoc* and bacteria seemed to have no appreciable effect on the quantity of nitrogen fixed. Bouilhac thus concluded that *Schizothrix lardacea* and *Ulothrix flaccida* (either alone or in the presence of soil bacteria) and *Nostoc punctiforme* (in the pure state) are unable to fix free atmospheric nitrogen in the absence of combined nitrogen. The abundant fixation in the cultures containing a mixture of *Nostoc* and soil bacteria is not ascribed by the author to the activity of either organism alone.

Richter (28) observed pots of soil with and without plants, some placed in the dark, others in the light. While a rich algal vegetation developed in the latter, none appeared in the former. Only in a few cases was the growth accompanied by a marked free-nitrogen fixation, but in these instances the author believes it due to the algæ. Pure cultures were not employed. Benecke (1) contributed some observations made on cultures of *Hormidium*, *Vaucheria*, *Cladophora*, and members of the *Conjugales*,—all containing bacteria. In nitrogen-free cultures there appeared what Benecke termed "nitrogen-hunger," a condition which is characterized in *Hormidium* by the production of very long, pale filaments, the cells of which become extremely long and in which the development of the chloroplast is so meager that the cells are almost colorless. Stocklasa (36) found that the "Alinit" bacteria fix free gaseous nitrogen in much larger quantities when grown in the presence of species of *Stichococcus* and *Nostoc*. This influence he considers to be due to the pentosans which, according to his belief, are present in large quantities in various algæ, and which, because of their ready solubility in water, serve as a favorable energy-furnishing medium for free-nitrogen-fixing bacteria.

A noteworthy contribution to the subject is that of Krüger and Schneidewind (19). These authors for the first time conducted extensive experiments with a variety of algæ in pure culture, including *Stichococcus chloranthus*, *S. major*, *S. bacil-*

laris, and *S. sp.*, the latter isolated from five different sources; *Chlorella sp.*, from the group of which *Chlorella vulgaris* Bey. is typical (also isolated from five different localities); *Chlorella protothecoides* and three other isolations of a form or forms belonging to the same group; *Chlorothecium saccharophilum* and five other isolations of forms belonging to the same group; and lastly, *Cystococcus humicola*. The media employed by the authors included the following:

1. One per cent dextrose, 0.2 per cent K_3PO_4, 0.04 per cent MgSO4, 0.02 per cent $CaCl_2$, and 1 drop of a 2 per cent $FeCl_3$ solution to each 100 cc. of solution.
2. Ignited sand moistened with solution 1.
3. Solution 1 plus 0.25 per cent $(NH_4)_2SO_4$, and 0.25 per cent $NaNO_3$.
4. Ignited sand moistened with solution 3.
5. One-half per cent beef extract, ½ per cent peptone, and ½ per cent dextrose.
6. Ignited sand moistened with solution 5.
7. Diluted beerwort.
8. Ignited sand moistened with solution 7.
9. Humous clay soil plus 35 per cent sand moistened with distilled water.

The results obtained were uniform in that the media, free from combined nitrogen, failed to produce a healthy growth, whereas those containing nitrogen in a combined form showed an abundant growth,—some of the algæ preferring the nitrogen in an organic and others in an inorganic form. Further, no fixation of free atmospheric nitrogen was noted in any of the cultures. Krüger and Schneidewind conclude that there is a strong probability that all other chlorophyllous soil algæ of this kind are unable to fix free atmospheric nitrogen, and, in general, agree with the opinion of Kossowitsch that the soil-inhabiting algæ supply the free-living, nitrogen-fixing organisms with the necessary non-nitrogenous, energy-furnishing material.

Conclusions similar to those of Kossowitsch were reached by Deherain and Demoussy (8), who succeeded in cultivating blue lupines free from root nodules in humus-free sand, the surface of which became covered with *Phormidium autumnale* and *Ulothrix flaccida* in the course of the experiments. The authors

explained the growth of the lupines by supposing that the soil bacteria fixed free nitrogen at the expense of energy-furnishing organic materials supplied by the algæ, and that the nitrogen so fixed in organic form became available to the legumes.

A return to the conclusion that members of the *Cyanophyceæ* fix free atmospheric nitrogen is found in an investigation by Beyerinck (2). From 1½ to 2-liter portions of tap or distilled water containing 0.02 per cent dipotassium acid phosphate were inoculated with 1–2 grams of garden soil, and placed in the light. After several weeks a characteristic growth of blue-green algæ developed, containing, among other species, *Anabæna catenula,* a form related to or identical with *Nostoc paludosum,* and *Nostoc sphæricum,*—all non-motile species of *Cyanophyceæ.* The development of the blue-green algæ in an almost nitrogen-free medium led Beyerinck, without analytical data, and in spite of the evident contamination of his cultures with soil bacteria, to the conclusion that the *Cyanophyceæ* belong to the class of organisms possessing the faculty of free-nitrogen fixation. He regards the *Cyanophyceæ* as the only known organisms capable of synthesizing their organic materials from carbon dioxide and free nitrogen, and considers as significant in this connection the observations of Graebner (13) and Treub (37), who found that in the sequence of floras on fresh sand and lava soils, species of *Cyanophyceæ* are the first to appear.

Cystococcus humicola was once more subjected to a careful investigation by Charpentier (7). His previous experiments had demonstrated that the dry weight of algal growth obtained in liquid glucose media was about one-half that of the weight of glucose consumed, and that 5.14 per cent of this dry weight was nitrogen. He then pointed out that the quantity of nitrogen furnished by Kossowitsch to his pure cultures of *Cystococcus humicola* in the form of potassium nitrate was sufficient to produce at least 40 milligrams of growth (dry weight), and that while this growth was being produced it might not be necessary for the alga to seek nitrogen from the atmosphere. Once the dextrose was exhausted, the alga might, it is true, develop at the expense of atmospheric carbon dioxide, but the author holds the opinion that this would mean a double expenditure of energy for the assimilation of both carbon dioxide and free nitrogen and

that under these conditions growth would be difficult. Because of the vast amount of energy necessary for free-nitrogen fixation, as illustrated by *Clostridium Pasteurianum*, the author suggests that there is a strong probability that *Cystococcus* is capable of assimilating free nitrogen only when the expenditure of energy in carbon assimilation is reduced to a minimum,—that is to say, when abundant available organic materials are furnished. He further emphasizes the necessity of employing combined nitrogen in a less readily available form than nitrates, suggesting organic nitrogenous compounds. On media composed of a decoction of beans to which were added 1 per cent and 2 per cent of dextrose and gelatin, respectively, *Cystococcus* was grown and the entire culture analyzed for total nitrogen. Although care was taken to have an abundance of available organic material (dextrose) present, Charpentier found that in no case was there any indication of free-nitrogen fixation. He further found that ammonia, asparagin, and peptone were each able to serve as the sole source of nitrogen.

The association of blue-green algæ and soil bacteria is again referred to as an effective agent in free-nitrogen fixation by Bouilhac and Giustiniani (5, 6). Buckwheat, white mustard, corn, and cress were planted in clean sand moistened with a mineral nutrient solution free from combined nitrogen, and the substrata inoculated with *Nostoc punctiforme* and *Anabæna sp.* covered with bacteria. The phanerogams grew to maturity and analysis showed a marked fixation of free atmospheric nitrogen.

Of particular interest are the observations of Heinze (14), who, however, fails to state whether or not the *Chlorella* experimented with was in pure culture. He found that no appreciable growth took place in cultural solutions free from combined nitrogen, but that in the presence of the latter a rich growth appeared, unaccompanied, however, by a definite fixation of nitrogen. More important are his experiments with *Nostoc* in impure condition, a good growth of the form being obtained in a mineral nutrient solution free from combined nitrogen and sugar. These cultures, as well as others on soil inoculated with a similar *Nostoc* culture contaminated with bacteria and fungi, showed a definite amount of free-nitrogen fixation. Heinze was unable to find *Azotobacter* present, and this, together with the observa-

tion that the contaminating fungus in pure culture was unable to fix free nitrogen, led the author to the conclusion that the *Nostoc* is, in all probability, directly responsible for the free-nitrogen fixation. Further, he would place *Azotobacter* in close relationship with the *Chroococcaceæ*, a family in which, he suggests, some forms capable of fixing free atmospheric nitrogen may be found.

Richter (29), working with pure cultures of *Nitzschia palea* and *Navicula minuscula*, reached the conclusion that the former, and probably the latter also, is unable to assimilate elementary nitrogen in the absence of combined nitrogen. Heinze (15), in experimenting with a *Nostoc* culture which he had purified until it contained as a contamination only a *Streptothrix*, found that in solutions free from combined nitrogen and sugar but containing respectively mono, di, and tripotassium phosphate, a clearly demonstrable amount of free atmospheric nitrogen was fixed. The *Streptothrix* was subsequently isolated and tested as to its ability to fix elementary nitrogen, both with and without sugar, but always with negative results. In conclusion, Heinze reasserts his former belief that *Nostoc* is capable of fixing elementary nitrogen.

Mameli and Polacci (22) succeeded in growing *Oedogonium*, *Spirogyra*, *Zygnema*, and *Protococcus* in nutrient solutions free from combined nitrogen, and demonstrated by analysis an increase in total nitrogen. They ascribed to these forms, and to chlorophyllous cells in general, the faculty of synthesizing ammonia from free nitrogen and nascent hydrogen. Pure cultures were not used. Boresch (3) found that *Phormidium corium* Cohn became brown when grown in solutions containing very small amounts of combined nitrogen, but that the green color reappeared following the addition of potassium nitrate or organic nitrogen compounds. Several species of *Oscillatoria*, *Rivularia*, and *Chroococcus* behaved similarly. But *Anabæna sp.* did not change color even when the solution in which it was growing had become completely exhausted of its combined nitrogen. While the investigation concerns itself primarily with the relation of nitrogen to the color in algæ, the observations point once more to species of *Anabæna* as possibly belonging to the class of free-nitrogen-fixing organisms, and

equally clearly to the conclusion that the remaining forms experimented with do not belong to this class.

Oes (25) made the observation that *Azolla* with its endophytic *Anabæna Azollæ* grew exceedingly well in mineral nutrient solution free from combined nitrogen. Analysis showed a distinct fixation of nitrogen. Attempts to cultivate the *Anabæna* in pure culture failed. While calling attention to the possible direct rôle of the associated bacteria in the observed fixation, the author inclines to the view that *Anabæna Azollæ* is itself capable of fixing free atmospheric nitrogen.

The preceding survey of literature shows that in all of the earlier investigations, and in a considerable number of the later ones, impure cultures were used. In experiments conducted under these conditions, it is evident that negative results are, in general, more reliable than positive ones. Attention should therefore be called to the negative results which have been obtained from investigations with impure cultures. These, as will be seen from the literature cited, include a large number of genera and species from both grass-green and blue-green algæ, and indicate in many cases with a reasonable degree of certainty that the faculty of elementary-nitrogen fixation is absent in a very considerable number of species of both the *Chlorophyceæ* and *Cyanophyceæ*. In the former class, all investigations conducted with pure cultures have led without exception to the conclusion that these forms are unable to fix free atmospheric nitrogen.

As regards the *Cyanophyceæ*, it should be stated at the outset that while many observations are on record both affirming and denying free-nitrogen fixation in the group, it is questionable whether experiments have been conducted with more than a single species in pure culture. Bouilhac, it is true, claims to have isolated *Schizothrix lardacea* and *Nostoc punctiforme* in pure culture. From the meager account given of the isolation technique, it appears very improbable that the latter form was actually obtained in culture free from bacteria, although the former may have been. However, the work of Heinze, while not conducted with pure cultures, renders free-nitrogen fixation in *Nostoc* probable, and it appears especially desirable, there-

fore, to study representatives from this genus as well as other members of the group *Cyanophyceæ*.

In the progress of the work about to be reported, it soon became obvious that the development of pure culture methods would constitute a very considerable portion of the investigation, and it was deemed advisable to limit the nitrogen phase of the problem to the fixation of atmospheric nitrogen in the complete absence of combined nitrogen, leaving for a subsequent report the problem of elementary-nitrogen fixation in the presence of combined nitrogen. The work concerning pure culture methods will be found reported elsewhere (34).

Experimental

Materials and apparatus

Most of the algæ isolated were soil-inhabiting species. Preliminary experiments showed that in nearly every case better growth was obtained on solid media than in liquid ones. For this reason it was decided to conduct all experiments concerning the fixation of free nitrogen in the complete absence of combined nitrogen on a solid medium. Agar could not be sufficiently freed from all traces of combined nitrogen, and the difficulties involved in preparing large quantities of silicic acid jelly sufficiently pure were so great that a No. $2\frac{1}{2}$ ground quartz was finally decided upon.

Preparation of the Sand.—The sand, after being thoroughly washed, was boiled in concentrated hydrochloric acid for two hours, subsequently washed free from chlorides with distilled water, and then heated almost to dull redness for from four to five hours. The sand was then boiled a second time in chemically pure concentrated hydrochloric acid and again washed with distilled water until chlorides could no longer be detected. When this stage had been reached the washing with distilled water was continued a dozen times more, after which the sand was drained as thoroughly as possible and the washing completed with from five to ten changes of nitrogen-free water. After drying the sand in a clean evaporating dish, a sample was boiled in nitrogen-free water and the liquid tested for ammonia, nitrites and nitrates, but only uniformly negative results were obtained.

Nitrogen-free Water.—The distilling apparatus used was, in general, like that described by Jones and Mackay (16) for the preparation of water with a very low electrical conductivity, except that the water was triply distilled in place of doubly, and from glass throughout in place of being condensed in a block tin tube. Fig. 1 represents the distilling apparatus, and it need only be pointed out that flask III was added to obviate any possibility of contaminating the distillate with spray from flask II. The water obtained from this still gave uniformly negative results when tested for ammonia, nitrites and nitrates.

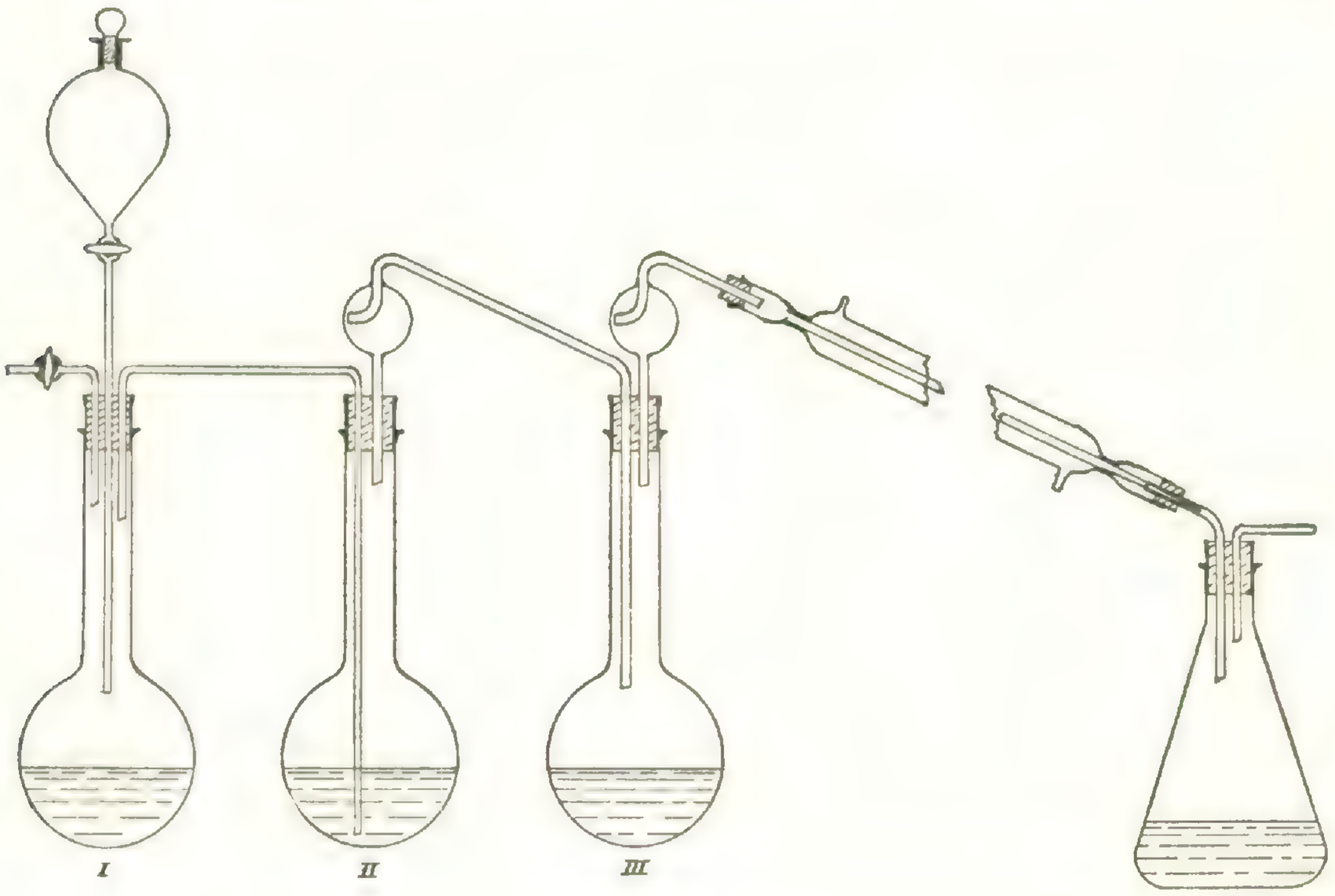

Fig. 1. Distilling apparatus for nitrogen-free water

Cultural Apparatus.—One hundred cc. flasks, carefully cleaned in acid-dichromate cleaning mixture, rinsed in nitrogen-free water and dried, were connected in series of ten each in the 1912 experiment (eight in the 1913 experiment) by means of glass tubing and rubber stoppers as shown in pl. 3 fig. 1. The glass tubing was cleaned in the same manner as the flasks, and the rubber stoppers were boiled in dilute alkali, then in dilute hydrochloric acid, and subsequently washed with distilled and nitrogen-free water. Into each flask of the 1912 experiment an accurately weighed 40-gram quantity (in the 1913 experiment

30 grams) of sand was placed. For purposes of aëration the separate series of flasks were joined together in groups of five, as shown in pl. 3 fig. 2, and the free end of the common connecting tube provided with three sets of triple wash-bulbs, —the two nearest the flasks containing nitrogen-free water, which served to moisten the air after passing through the third bulb containing 25 per cent sulphuric acid. In order to aërate any particular series of flasks it was only necessary to attach a filter pump to the rubber tube at the end of the series which it was desired to aërate and to open the pinchcock until the desired stream of air passed through the wash-bulbs.

Chemicals.—The inorganic compounds used were all Baker and Adamson's analyzed chemicals; the organic compounds were Merck's highest purity chemicals.

Cultural Solutions.—In the 1912 experiment, in which each series contained ten flasks, the following ten cultural solutions were used and in the following order, the flasks being numbered correspondingly:

1. NH_4NO_3 0.5 grams,
   $MgSO_4.7H_2O$ 0.2 grams,
   K_2HPO_4 0.2 grams,
   $CaCl_2.?H_2O$ 0.1 grams,
   $FeSO_4$ trace,
   Nitrogen-free water 1000 grams.
2. The same as No. 1, but containing 0.250 grams of NH_4NO_3 instead of 0.5 grams.
3. The same as No. 1, but containing 0.100 grams of NH_4NO_3 instead of 0.5 grams.
4. The same as No. 1, but containing 0.050 grams of NH_4NO_3 instead of 0.5 grams.
5. The same as No. 1, but free from combined nitrogen.
6. The same as No. 5, but containing 2 per cent *d*-glucose.
7. The same as No. 3, but containing 2 per cent *d*-glucose.
8. The same as No. 5, but containing 2 per cent mannite.
9. The same as No. 3, but containing 2 per cent mannite.
10. The same as No. 3, but containing 2 per cent saccharose.

In the 1913 experiment, in which each series contained eight flasks, the following eight cultural solutions were used:

1. The same as No. 5 in the 1912 experiment.
2. The same as No. 1 in the 1912 experiment.
3. The same as No. 5 in the 1912 experiment, but with 5.26 grams of *d*-glucose (making a glucose solution isotonic with a 1 per cent saccharose solution) added.
4. The same as No. 1 in the 1912 experiment, but with 5.26 grams of *d*-glucose (making a glucose solution isotonic with a 1 per cent saccharose solution) added.
5. The same as No. 5 in the 1912 experiment, but with 5.32 grams of mannite (making a mannite solution isotonic with a 1 per cent saccharose solution) added.
6. The same as No. 1 in the 1912 experiment, but with 5.32 grams of mannite (making a mannite solution isotonic with a 1 per cent saccharose solution) added.
7. The same as No. 5 in the 1912 experiment, but with 10.00 grams of saccharose (making a 1 per cent saccharose solution) added.
8. The same as No. 1 in the 1912 experiment, but with 10.00 grams of saccharose (making a 1 per cent saccharose solution) added.

The solutions containing the organic compounds were all made isotonic in order to obviate possible differences in growth due to different osmotic pressures of the cultural solutions.

The exact volume of solution necessary to just saturate the amount of sand used in each flask was determined and this amount of the various solutions added to the corresponding flasks. The stoppers were then lightly inserted and one group sterilized at a time in a large Kny-Scheerer horizontal autoclav, at six pounds pressure for one and one-quarter hours.

INOCULATION

The groups of flasks were transferred directly from the autoclav to the inoculating room which had previously been "steamed down." All inoculations were made with a DeVilbis atomizer, which, with the exception of the bulb, is made of metal and glass throughout. The bulb was removed and the opening of the metal tip, to which the former is attached, plugged with cotton. After filling the glass container one-half full of solution No. 5 (1912 series), the whole (with the exception of the bulb)

was sterilized. After cooling, the liquid was inoculated with the desired organism (care being taken to avoid introducing any agar, which can readily be done if hard agar cultures are used from which to make the inoculation). The DeVilbis atomizer is provided with an adjustable metal tip so that the spray may be directed downward. The metal tip further admits of sterilization by flaming. By exercising care and keeping the hands moist with alcohol, comparatively few contaminations result, only four having appeared in a total of 320 inoculations.

Attention should be called to the importance of inoculating in such a way that an approximately equal number of organisms are introduced and that they are uniformly distributed over the substratum. Unless this is done growth comparisons cannot be made with any considerable degree of accuracy, as differences may be due to localized and unequal inoculation. This is especially true in algæ which do not form motile cells and which, therefore, are unable to spread rapidly over the substratum.

GROWTH AND OBSERVATIONS

All groups of flasks of the 1912 experiment were placed in the light of north windows at the ordinary room temperature and the cultures aërated at intervals of from three days to a week.

The 1913 experiment was set up in duplicate, one-half being placed in a glass incubator kept constantly at from 29.5 to 30.5° C., and the other half in a similar incubator at the ordinary room temperature. Both series of cultures were placed directly in front of a north window and were aërated from time to time.

Space will not permit the detailed tabulation of the observations on growth. In the following tables, growth is indicated without reference to time. A few general statements may, however, serve to give some idea as to the relation of the composition of the cultural medium to the time elapsing before a macroscopically visible growth appeared. In almost every case, growth was observed first on the glucose-containing medium and almost as soon or slightly later on the one containing saccharose. It should be said, however, that a healthy growth was maintained on these two media, in most cases, for but a short time. *Chlamydomonas pisiformis* Dill forma *minor* Spargo is a marked exception in this respect, a splendid, healthy

TABLE II

RESULTS OF THE 1912 EXPERIMENT

(August 6, 1911—July 1, 1912.)

Organism	Sol. 1* (with comb. nitrogen)	Sol. 5 (without c.n.)	Sol. 6 (without c.n.)	Sol. 7 (with c.n.)	Sol. 8 (without c.n.)	Sol. 9 (with c.n.)	Sol. 10 (with c.n.)
Chlamydomonas pisiformis Dill forma *minor* Spargo	++	−†	−	++++	−	++++	++++
Chlorella sp., large form with clathrate chromatophore	+++	−	−	++	−	+++	++
Kirchneriella sp., a form without marked gelatinous envelope	+	−	−	+	−	+	+
Protosiphon botryoides (Kütz.) Klebs	++	−†	−†	+++	−	+++	+++
Chlorococcum humicola (Näg.) Rabenh.	+++	−	−	++++	−	+++	++++
Chlorella vulgaris Bey.	+++	−†	−	++	−	+	++
Stichococcus bacillaris Näg.	+++	−	−	++	−	−	−

Slight, fair, good, and splendid growths are respectively indicated by +, ++, +++, and ++++. No growth is indicated by −.

* The mention of growth in solutions 2, 3, and 4 is omitted because growth differences were not marked. These solutions were introduced in each series in order to note the effect of steadily decreasing quantities of ammonium nitrate on the intensity of growth.

† A scarcely detectable growth developed in these cases which, however, disappeared in all cases within a short time. The growth was so slight as to be noticeable only when the flasks were compared with others absolutely free from growth.

growth being maintained for a very long time. Growth on the mannite-containing medium was usually slower in making its appearance, but, in general, remained healthy for a longer period of time than those on glucose or saccharose. With very few exceptions, growth appeared last on the purely synthetic medium, but was maintained in a state of vigor longer

TABLE III

RESULTS OF THE 1913 EXPERIMENT

(March 29–April 16, 1913.)

Organism	Sol. 1 (without c.n.)	Sol. 2 (with c.n.)	Sol. 3 (without c.n.)	Sol. 4 (with c.n.)	Sol. 5 (without c.n.)	Sol. 6 (with c.n.)	Sol. 7 (without c.n.)	Sol. 8 (with c.n.)
Chlamydomonas pisiformis Dill forma *minor* Spargo. Temp. 18.5–24°C.	–	++	–	+++	–*	++	–	+++
Ditto. Temp. 29.5–30.5°C.	–	–	–	++	–	–	–	++
Chlorella sp., large form with clathrate chromatophore. Temp. 18.5–24°C.	–	+	–	++	–	+	–	+++
Ditto. Temp. 29.5–30.5°C.	–	+	–	++	–	–	–	++
Stichococcus bacillaris Näg. Temp. 18.5–24°C.	–	+	–	++	–	+	–	++
Ditto. Temp. 29.5–30.5°C.	–	–	–	++	–	–	–	++
Chlorococcum humicola (Näg.) Rabenh. Temp. 18.5–24°C.	–	+	–	++++	–	+	–	++++
Ditto. Temp. 29.5–30.5°C.	–	–	–	+++	–	–	–	+++
Protosiphon botryoides (Kütz.) Klebs. Temp. 18.5–24°C.	–	+	–	+++	–	++	–	+++
Ditto. Temp. 29.5–30.5°C.	–	–	–	+++	–	–	–	+++
Chlorella vulgaris Bey. Temp. 18.5–24°C.	–	+	–	++	–	+	–	++
Ditto. Temp. 29.5–30.5°C.	–	–	–	–	–	–	–	–
Kirchneriella sp. Temp. 18.5–24°C.	–	–	–	–	–	–	–	–
Ditto. Temp. 29.5–30.5°C.	–	–	–	–	–	–	–	–

*A scarcely detectable and rapidly disappearing growth developed as in the 1912 experiment.

than on any of the organic-compound-containing media. Glucose, saccharose, and mannite were chosen as energy-furnishing compounds because of their general usefulness in this capacity among free-nitrogen-fixing bacteria, and also because they are representatives from three great classes of carbon compounds.

Certain unpublished experiments, carried out by B. M. Duggar on nitrogen fixation in the fungi, indicate that, whereas no fixation takes place at ordinary temperatures, it does take place at elevated temperatures. It was thought desirable, therefore, to investigate the effect of elevated temperature on the process of elementary-nitrogen fixation by algæ in the absence of combined nitrogen. However, the results tabulated in table III show clearly that not only did no growth on any nitrogen-free medium appear at the higher temperature, but also that that appearing on nitrogen-containing media was, in many cases, poorer than that obtained in cultures kept at ordinary temperatures. It should further be noted that growth was in some cases entirely suppressed. It would appear, therefore, that in the species investigated, growth at elevated temperatures is less vigorous than at ordinary temperatures and that, in all probability, no favorable effect on free-nitrogen fixation is to be expected by growing these species at the higher temperature maintained in the experiment.

The incipient, ephemeral growth which was observed in a few cases where combined nitrogen was not furnished is believed to be due to the minute quantity of combined nitrogen which was unavoidably introduced in the inoculation process. The inoculating material was, of necessity, derived from agar containing ammonium nitrate, and while no agar was transferred it is altogether probable that enough combined nitrogen was carried over in the water adhering to the cells to account for the trace of growth. It should be emphasized again that in every case this growth was so slight as to have escaped detection had not a comparison been made with a flask absolutely free from growth.

In table IV the results of the two experiments are combined and show that in seven species complete results have been obtained. These results indicate with perfect uniformity that growth, under the conditions realized in the experiments, is impossible in the absence of combined nitrogen, even when readily

TABLE IV

SUMMATION TABLE OF 1912 AND 1913 EXPERIMENTS

(Solutions numbered as in 1913 experiment.)

Organism	Sol. 1 (without c.n.)	Sol. 2 (with c.n.)	Sol. 3 (without c.n.)	Sol. 4 (with c.n.)	Sol. 5 (without c.n.)	Sol. 6 (with c.n.)	Sol. 7 (without c.n.)	Sol. 8 (with c.n.)
Chlamydomonas pisiformis Dill forma *minor* Spargo	−	++	−	++++	−	++++	−	++++
Chlorella sp.	−	+++	−	++	−	+++	−	+++
Stichococcus bacillaris Näg.	−	+++	−	++	−	+	−	++
Chlorococcum humicola (Näg.) Rabenh.	−	+++	−	++++	−	+++	−	++++
Protosiphon botryoides (Kütz.) Klebs	−	++	−	+++	−	+++	−	+++
Chlorella vulgaris Bey.	−	+++	−	++	−	+	−	++
Kirchneriella sp.	−	+	−	+	−	+	−	+

assimilable energy-furnishing compounds like glucose, mannite, and saccharose are supplied; and that, therefore, these forms, under the conditions stated, are totally unable to fix free atmospheric nitrogen in the complete absence of combined nitrogen.

Conclusions

1. In agreement with all work that has previously been done on the assimilation of elementary nitrogen by grass-green algæ in pure culture, it has been found that *Chlamydomonas pisiformis* Dill forma *minor* Spargo, *Protosiphon botryoides* (Kütz.) Klebs, *Chlorococcum humicola* (Näg.) Rabenh., *Chlorella vulgaris* Bey., *Stichococcus bacillaris* Näg., *Chlorella sp.*, and *Kirchneriella sp.*, are unable to fix free atmospheric nitrogen in the complete

absence of combined nitrogen, under the conditions realized in the experiments.

2. A slightly elevated temperature (from 5 to 10° C. above the ordinary range of room temperature—18–24°C.) does not, as is the case in certain fungi, enable the algæ investigated to fix free gaseous nitrogen in the complete absence of combined nitrogen.

In conclusion, the author wishes to express his sincere appreciation and gratitude to Dr. George T. Moore, at whose suggestion the work reported upon in this paper was undertaken and under whose constant attention and generous aid it was carried to completion; to Dr. B. M. Duggar, for many valuable suggestions and innumerable courtesies; to Mildred Spargo Schramm, for kindly encouragement and help throughout the investigation; and to Dr. George R. Hill, Jr., for substantial aid during the last year of the work.

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Explanation of Plate

PLATE 3

Fig. 1. Culture flasks containing quartz sand joined together in series of eight each, but before arrangement into groups.

Fig 2. Five series of culture flasks arranged in a group with a common connecting tube (on the left) and a series of three triple wash-bulbs. On the right, the rubber tubing, provided with pinchcocks, is shown attached to each series for use in aëration.

FIG. 1.

FIG. 2.

COCKAYNE, BOSTON

THE THELEPHORACEÆ OF NORTH AMERICA. I[1]

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Introduction

This monographic study of the North American *Thelephoraceæ* was begun in 1894 as the author's contribution towards a greatly needed manual of the *Basidiomycetes* of the United States,—a need that still confronts us. It has been necessary to carry on these investigations in connection with college and other work which required most of my time, but the long period covered has been an advantage; for during these two decades there has been such widespread interest in the *Thelephoraceæ* on the part of American students of fungi that it has been possible to study this family and its distribution from extensive series of freshly collected specimens from all the important regions of North America with the exception of Alaska, Mexico, and the Colorado-New Mexico region of the United States, from which but small collections have been received. These specimens have been preserved unpoisoned in my herbarium in insect-proof tin boxes which receive herbarium sheets, and each will be cited by the number or other designation adopted by my correspondents in order that their specimens may be as useful for future reference as my own. The quantity of material always awaiting examination has confined my work to a systematic treatment of this family.

Except in the case of types of species, specimens of published exsiccati, and the specimens of Schweinitz's herbarium, I cite but few specimens from the large herbaria. This is done on account of the difficulty and large amount of time involved in making a study of the material contained in them. Serious changes in the condition of the specimens in these herbaria have been occasioned partly by time but more largely by the poisonous solutions with which the specimens were soaked for preservation under old-fashioned methods of herbarium procedure,—

[1] Issued July 1, 1914.

methods well enough adapted for flowering plants but not for fungi.

Early in the work it became apparent that the diagnoses of known species of resupinate *Thelephoraceæ* had failed utterly to enable the leading working mycologists of any country to recognize with certainty in the species about them those described in other countries, or those described for their own country by earlier students. The truth of this statement is shown by the errors and confusion in names of the common species which have been distributed in exsiccati, by the fact that in the large herbaria several different species are likely to bear the same specific name on the same or successive sheets, and by the vastly more important fact that the masters of mycology of each age, when relying wholly on the diagnoses published by their contemporaries or predecessors, have described as new species common and conspicuous resupinate fungi which had been accurately described by immediate contemporaries or predecessors, and in very many cases just as accurately by still earlier students. All the mycologists concerned in these redescriptions have been earnest strivers after truth, I am convinced, and would have preferred to employ the earlier names for their plants, could they have known that those earlier names referred with certainty to their specimens. All these people were relying, as was the usage of their time, on a few words of published description in some other than their mother tongue.

It is time to recognize generally that the resupinate *Hymenomycetes*, and especially the *Thelephoraceæ*, are extremely difficult taxonomic problems. Descriptions must include more than a rather vague and generalized characterization of the mere superficial appearance and habit of the specimen with possibly a reference to spores which some one recorded for what was perhaps this species. The fungus itself is an individual of the species; the description in words and by illustration has merit in proportion to the success it has in producing in the mind of any educated stranger exactly the ideas which he could derive from the study in detail of the specimen itself. From the specimen, exact ideas may be had of coloration, of form, of dimensions, of texture, of consistency, of internal structure, of organs of minute size, of place of growth, and of host and

substratum. If the description fails to give the color as exactly as if it had been noted by comparison with such a standard work as Ridgway's 'Color Standards' or Saccardo's 'Chromotaxia,' then it is inferior to the specimen; if the description contains no information as to whether the basidia are simple or cruciate, making up the whole hymenium or arranged side by side with other organs of characteristic form, standing directly on the substratum or separated from it by densely or loosely interwoven hyphæ or other form of subhymenial layer;—if it does not contain all this information in exact terms and as much in addition as the specimen itself could afford, then it is an imperfect description of the species. It may be so imperfect that a dozen different species of fungi could be assembled, to any one of which it would apply as well as to any other, as is the case with the supposedly common and cosmopolitan *Corticium lacteum* and *C. calceum*. Published exsiccati probably contain the full dozen under each of these names.

In the case of resupinate *Hymenomycetes*, types and authentic specimens of the species are of the highest importance to supplement the prevailingly imperfect descriptions with full and exact data. Hence, the types of fungi on which the descriptions are based and the authentic specimens from the authors of the species are of importance in proportion to the degree in which these plants may yield data not afforded by the descriptions and existing illustrations of the species. In the case of the resupinate *Hymenomycetes*, the early descriptions are of slight practical value except as they are backed up by types and specimens from their authors. For this reason, if there had been no other, the International Botanical Congress, at Brussels, acted for the best interests of mycology in fixing the beginning of the naming of *Hymenomycetes* with the publication of Fries' 'Systema Mycologicum,'—the time when the preservation of types and authentic specimens of such fungi in herbaria became so prevalent that it was possible for later mycologists to distinguish the resupinate species by taking the trouble to study the types, if authentic specimens could not be obtained.

My method of becoming acquainted with our described species of *Thelephoraceæ* has been to study and arrange by species in my herbarium the specimens as they have accumu-

lated. In this arrangement due regard has been given to original descriptions of species and to all details of internal structure. Spore collections on glass slides have been made for each species whenever possible, and about five thousand mounts of sectional preparations in glycerin have been made from collections and preserved for reference in connection with internal structure of the specimens. From time to time I have taken my *Thelephoraceæ* to herbaria where the types of our American species are stored and have there painstakingly matched them with the types. I have made sectional preparations from a fragment of each of these types in order to make sure that my specimens match the types not only in external characters but also in all details of internal structure. The sectional preparations of type specimens have been preserved in glycerin. Specimens from my herbarium which have been so matched with type specimens have been used by me later for the determinations of subsequent collections. Such methods of investigation are probably too laborious and require too much time to become popular and they afford little opportunity for the inspirational flights attributed to genius, but they do afford a means of determining within very narrow limits the species of North American *Thelephoraceæ*.

I am under especial obligation to Dr. W. G. Farlow for suggesting this work, for interest in its progress, and for frequent access to the Curtis Herbarium for comparisons with types. I am indebted also to Dr. C. H. Peck for opportunity to study his types in the New York State Herbarium, to the late Dr. L. M. Underwood for similar opportunity with the Ellis types in the Herbarium of the New York Botanical Garden, to Dr. S. W. Dixon and Professor S. Brown, of the Philadelphia Academy of Natural Sciences, for the privilege of studying in the Schweinitz Herbarium, to Sir W. T. Thistleton-Dyer and Mr. G. Massee for access to types and authentic specimens in Kew Herbarium, to the late Dr. T. M. Fries for the privilege of studying in the Herbarium of Elias Fries, at Upsala, and to Mr. Lars Romell, of Stockholm, Dr. P. A. Karsten, of Mustiala, and Abate G. Bresadola, of Trient, for many authentic specimens of their own species and for specimens which they had compared with types of early authors of *Thelephoraceæ* of

Europe. In the later pages names of the many botanists who have participated in this work by the contribution of specimens from their respective regions are given in connection with the specimens. I feel my obligation to each of these correspondents.

Having become thoroughly familiar with the species of a family of fungi, one then faces the task of deciding under what genera they shall be grouped in order that others may more easily recognize them. Our studies in systematic botany and the accumulations of plants in herbaria are primarily for the purpose of enabling those who wish to obtain information about any particular plant, however obscure, to determine its name accurately and so be in a position to get at the world's literature and knowledge concerning that species; and also to enable botanists so to entitle and index their researches that the results will be more available to the world at large. Stability in the nomenclature of plants is therefore important, and revolutionary changes in generic conceptions should not be lightly and frequently made. Whenever one proposes new genera to supersede a well-established genus which has satisfactorily embraced the related species of the world, the burden of proof should be on the one who makes the change to demonstrate that the advantages from the innovation will more than compensate for the confusion which would result as well as for the loss of knowledge indexed under the superseded name.

Many new genera of fungi have been proposed during recent years. These have frequently come from students with a limited knowledge of the species of the world. It is not surprising that a botanist working on the few species of a limited region should be led to the establishment of new genera on the basis of what seem to be sharp differences in his species or groups of species. When, however, his knowledge encompasses just as definitely the structure of the many species of some large portion of the world, his perspective changes, and he may now find that the species which he formerly regarded as generically distinct are so closely connected by intermediate species that the contemplated generic separation would be unnatural and a hindrance to botanical progress. It is fundamental that genera be so sharply defined that any accurate observer who will make

the study necessary for the application of the generic definition may be sure ninety-nine times out of a hundred that the fungus on which he is working is a *Stereum*, for example, and not a *Thelephora*, nor a *Craterellus*, nor a *Cladoderris*, nor a *Corticium*, nor a *Peniophora*, nor a *Sebacina*. It is an obligation on authors to group their species so accurately under genera that *Stereum*, for example, shall comprise all the species of this genus known to science, and no others. The synonomy of species in later pages will show how vaguely the genera of *Thelephoraceæ* have been comprehended.

It is desirable that a genus should consist of but few species in those cases where the group is sharply and naturally set off from others, that is, where no intermediate species connect the genus with other groups. While such small genera are desirable, if wholly natural, it is in the highest degree objectionable to create small artificial genera by arbitrarily segregating the species of a natural genus and so establishing indefinite lines of demarkation between genera. Under such a procedure the generic location of certain species becomes wholly arbitrary and always continues as a stumbling block for new students and this leads to the loading of our literature with so-called new species. A case in point is Saccardo's scheme in the 'Sylloge Fungorum' in which he separates *Hypochnus* from *Corticium* and *Peniophora* without any natural generic planes of cleavage. In practical work one needs to know exactly what the generic limits of *Corticium*, *Peniophora*, and *Hypochnus* are. The question naturally arises as to just how loose and open the structure of the fructification must be to be included in the genus *Hypochnus* rather than in *Corticium* or *Peniophora*. Henning's violation of the principle involved is still more flagrant, for he separated the *Hypochnaceæ* as a new family from the *Thelephoraceæ*[1] and placed *Hypochnus* of Saccardo in the *Hypochnaceæ*, and *Corticium* and *Peniophora* in the *Thelephoraceæ*. As all students of the *Thelephoraceæ* have found *Hypochnus*, as understood by Saccardo, wholly unworkable, it would increase the usefulness of the 'Sylloge Fungorum' if Saccardo were to distribute among *Corticium* and *Peniophora*, the species which he now includes under *Hypochnus*.

[1]Engler und Prantl, Nat. Pflanzenfam. (I. 1**): 114. 1898.

Probably all species of *Corticium*, as originally understood, have an hymenium composed of basidia arranged side by side between non-sporebearing organs termed paraphyses. In many species, it is difficult to distinguish between the basidia and the paraphyses except by prolonged study of special preparations or by observations made at the time the basidia bear spores. In other species the sterile organs are conspicuous and distinct from the basidia either by their larger size, different form, or thicker or incrusted walls. Such conspicuous bodies are called cystidia, but if the paraphyses are merely finely but characteristically branched near their tips, they are not called cystidia. Such branched paraphyses occur in the hymenium of occasional species of several genera of the *Thelephoraceæ* and are valuable characters for specific diagnosis.

In 1880, Cooke proposed, from Kew Herbarium, to divide the old genus *Corticium* into two genera,—the name *Corticium* to be retained for those species having the non-sporebearing organs of the hymenium not distinguishable from the basidia, and the generic name *Peniophora* to be given to those species having cystidia. As the species of *Corticium* were very numerous and extremely difficult taxonomically, this proposal was hopefully received, and for more than thirty years the transfer of species from *Corticium* to *Peniophora* has been going on and the end has not been reached yet. During this long period there has been confusion as to which species of the old genus *Corticium* belong in the emended *Corticium* and which in the genus *Peniophora*.

Peniophora is an artificial rather than a natural genus, however, and its adoption has given to many species a position intermediate between this genus and *Corticium*. These intermediate species have to be classed with the one genus or the other according to personal judgment, for no one can state just how conspicuous the sterile organs must be, nor of how constant occurrence, to merit the name cystidia. In *Corticium Sambuci* Fr., for example, cystidia are readily found in preparations from some collections, but several preparations may have to be made to demonstrate them in other collections. In the same species and in different parts of the same section, cystidia may sometimes be sparingly and sometimes not at all incrusted. Some

species which I have placed in the genus *Peniophora* because of the presence of cystidia students may look for under *Corticium* when, by a more hasty study of their collections, they fail to detect these organs. On the other hand, students using more discriminating methods than mine may detect cystidia in species in which I have overlooked them, and such students will search in *Peniophora* for species which I have placed under *Corticium*. Species intermediate between genera always cause such trouble. There are many intermediates between *Peniophora* and *Corticium*, yet in this particular case the advantage from the separation undoubtedly more than compensates for the disadvantages occasioned by the intermediate species.

The case of *Peniophora* has been considered at length, because this genus is being regarded as a precedent for subdividing *Stereum* and grouping under *Lloydella* all those species which have conspicuous non-sporebearing organs between the basidia. Such a separation, however, would be artificial and give rise to a troublesome series of intermediate species, without the compensating advantage which accrued in the case of *Peniophora* and *Corticium*. *Stereum* is not a genus of difficult species nor does it comprise an immense number of species. It is just a fine, natural group of species capable of being more sharply defined than it was by Fries, so as to receive some species from *Thelephora* of Fries and to part with some to *Corticium*. So defined, even beginners will have no trouble in recognizing species of *Stereum*. Systematic work in mycology should strive to establish and maintain just such natural, clean-cut genera as *Stereum*.

It seems to me best to work along constructive rather than destructive lines. Fries had a wonderful ability for the perception of the natural grouping of fungi on the basis of gross morphology and habit. Since his time, research has greatly enlarged the knowledge of the internal structure of fungi and of the organs of propagation. The value of such organs in the classification of seed plants is well known. It is feasible to modify somewhat the genera of *Thelephoraceæ* as defined by Fries, in accordance with the true relationships and differences shown by the present knowledge of internal structure, basidia, and spores, and a system results which is the natural evolution of taxonomic and morphologic study of *Thelephoraceæ*. This

system has been communicated to my correspondents in connection with specimens. Its principal features are:

1. To restrict *Thelephora* to pileate species with simple basidia and colored spores.
2. To follow Karsten and Bresadola in placing under *Hypochnus* only resupinate species with colored echinulate spores.
3. To restrict *Stereum* to pileate species which have simple basidia and colorless spores and lack setæ in the hymenium.
4. To include in *Hymenochæte* all species having setæ.
5. To include in *Corticium* species always resupinate, which have colorless spores and lack cystidia, excepting those species which for other reasons are placed in *Exobasidium*. Include in *Corticium* hypochnoid as well as compact species.
6. To include in *Peniophora* all species which differ from *Corticium* merely by the presence of cystidia.

I find this system workable and very satisfactory for the accurate location of species in genera, except in the case of the species intermediate between *Peniophora* and *Corticium*. The proposals to subdivide *Peniophora* into *Glæocystidium, Peniophorella, Glæopeniophora*, etc., would create large numbers of species intermediate between the new genera, without compensating advantages.

I have studied the species of my predecessors and co-workers sympathetically and have endeavored to find real differences between their species and those previously known so that the validity of theirs might be confirmed. The great area of land covered by the present work, the differences in climate and substratum, and the keen search by my correspondents have brought to hand a very large number of specimens. I have earnestly striven to place them under species already known, but it has been necessary to describe many as new. I regret that there are so many of these. Should any one have reason to believe that in any case I have described as new a species already known, I shall esteem it a favor to receive an authentic specimen of the older species or to be informed where such a specimen can be consulted.

Colors of specimens were noted and recorded during the first years of my work by comparison with Saccardo's 'Chromotaxia' in accordance with his descriptive terms. Recently I have been using Ridgway's 'Color Standards and Nomenclature,' 1912, which has a greater variety of colors useful in the characterization of the species of *Thelephoraceæ*.

In my own work with collections of living fungi I am endeavoring to gather for each species a spore collection on a glass slip. The spores adhere well so that they may be covered by paper and preserved in the envelope with the dried specimens from which the spores were obtained. Such collections give the exact color and dimensions of mature spores. These dimensions are generally rather larger than those obtained from spores of sectional preparations of dried herbarium specimens. The spores of dried specimens, i. e., those remaining attached to the specimens, are probably too immature to be of normal size, and sometimes there are so few of them that one must exercise caution to avoid errors due to the study of spores foreign to the fungus.

Latex exists in many species of several of the genera and is more abundant and conspicuous in some species than in others, and its containing elements often extend to the hymenial surface. When specimens are in the vegetative condition, injury to the hymenium may liberate the fluid contents of the latex bodies so that this fluid exudes in colored drops at the edges of the wound, or discolors the bruised surface. For many of our species there is a lack of data concerning the color of this fluid or the discoloration. The latex bodies are pale brown in microscopic preparations made by my methods and must not be confused with setæ or cystidia. Latex is well shown in *Stereum spadiceum, S. sanguinolentum,* and *Corticium lactescens.*

There has been a disposition on the part of some authors to regard the *Thelephoraceæ* as not sharply separated from the *Hyphomycetes.* The specimens which I have collected, in striving to find all the *Thelephoraceæ* of my collecting region, and the specimens received from my correspondents afford no embarrassment in recognizing the most hypochnoid species of *Thelephoraceæ* by the basidia which characterize the families of *Hymenomycetes* in general.

The microscopical technique has been simplified as much as possible. Usually dried herbarium material had to be used for study and proved very satisfactory except in the case of specimens which had been subjected to poisoning processes for preservation in herbaria. A small bit of the fructification having a promising hymenial surface 2 or 3mm. square—but smaller if the specimen is a valuable type—is first moistened with alcohol, then wet with water and cut out from the rest of the specimen and from the substratum. This bit is then placed in a holder of elder pith and oriented so that the sections may be cut perpendicular to the surface of the hymenium and also contain as long hyphæ as possible. The sections are cut as thin as possible, free hand, with a very keen section razor flooded with alcohol. The thinnest sections are placed on a slide in a drop of water and then a drop of seven per cent aqueous solution of potassium hydrate is added.

Close observation of the sections should be made when the potassium hydrate solution comes in contact with them. For most species, the sections are merely cleared and the hyphæ swelled to the normal size of vegetative hyphæ. In a few species, the alkaline solution may dissolve out the color of the section on coming in contact with it, or it may change this color to a violet, which finally disappears, or it may cause disorganization changes in certain structures leading to their disappearance or destruction. Such changes should be observed and noted, for they are of help in the determination of the species. In the cases in which potassium hydrate solution exerts a destructive action, lactic acid should be employed with other sections in the manner described for potassium hydrate. Lactic acid clears and swells sections well, but so much more slowly than potassium hydrate that I have used it only where the latter is not satisfactory. After the sections have been cleared, the potassium hydrate should be drained off, the sections lightly stained on the slide with alcoholic solution of eosin (but not overstained), mounted in water, and studied at once.

For a thorough study of the species of the family at least one permanent preparation of each species should be retained for future comparisons. Permanent preparations may be made from the temporary water mounts by adding dilute glycerin—

two-thirds glycerin and one-third water—at the edge of the cover glass and allowing the glycerin to run under the latter as the water evaporates. When concentration of the glycerin is adequate, the excess should be wiped away with moist filter paper and the resulting smear removed to the very edge of the cover glass with a soft cloth moistened with 95 per cent alcohol. The preparations may then be sealed from the atmosphere by painting a ring of microscopical cement about the edge of the cover glass. At least two coats should be used for this ring, a light and very narrow one, and, after this dries, a very heavy, broad one. I have used Bell's Microscopical Cement, made in London, and Brunswick Black Cement. A variable percentage of the rings crack in the course of a few years and allow the glycerin to escape from under the cover glass, but the sections in such preparations can be remounted. Dr. Thaxter has very recently informed me that he has been using King's Transparent White Cement and King's Amber Cement for fifteen years and that none of the rings made with these cements have cracked. By the use of circular cover glasses rather than square ones, a microscopist's turn table may be used, thereby materially lessening the labor of preparing the rings.

SYSTEMATIC ACCOUNT

THELEPHORACEAE

Thelephoreæ Persoon, Myc. Eur. 1: 109. 1822; Fries, Hym. Eur. 629. 1872; Saccardo, Syll. Fung. 6:513. 1888.

Hymenomycetes with the hymenium inferior or amphigenous (on the lower surface or surrounding the fructification), coriaceous or waxy, even, rarely ribbed or papillate.

Through several of the genera the *Thelephoraceæ* connect closely with all the other families of the *Hymenomycetes*. *Hypochnus* approaches *Grandinia* of the *Hydnaceæ* in the granular hymenial surface of many of the species, but can be separated from this hydnaceous genus by the spore characters. *Lachnocladium*, with coriaceous structure, hairy stem, and colorless spores, is an intermediate genus between *Clavaria*, of the *Clavariaceæ*, and *Thelephora* but can be separated from the latter by the spore characters. *Craterellus* connects with

Cantharellus, of the *Agaricaceæ*. Some species of *Corticium* must be cautiously separated from *Merulius*, of the *Polyporaceæ*. The species of *Tremellodendron*, *Hirneolina*, and *Sebacina* were formerly distributed among *Thelephora*, *Stereum*, and *Corticium* respectively, but are now separated from these genera by the cruciate character of the basidia,—such basidia as are present in many *Tremellaceæ*. All these connecting genera will be included in the present monograph.

Michenera and *Heterobasidium* are excluded genera. Lyman has shown[1] that *Michenera artocreas* B. & C. is only a stage in the life history of *Corticium subgiganteum* B. & C., and that the genus *Michenera* has ceased to be a genus of the Basidiomycetes. My own study of the type of *Heterobasidium chlorascens* Massee, which is the type species of the genus, failed to locate any basidia whatever.

Very many *Thelephoraceæ* are of great economic importance on account of the dry rot induced by the growth of the mycelium in sills, floors, mine, bridge, and dock timbers, and other wooden structures located in moist, poorly ventilated places. *Coniophora puteana* is a common species which rots coniferous wood. Only a very few *Thelephoraceæ* are classed as serious plant parasites. Of these the rhizoctonial stage of *Corticium vagum* is the most important.

Key to the Genera

I. EU-THELEPHOREÆ:

Fructification not containing green lichen gonidia.

Fructification fleshy or membranaceous, often infundibuliform, with the hymenium distinct, continuous, even, ribbed or at length rugose; basidia simple ..... *Craterellus*

Fructification submembranaceous, cup-shaped, often pendulous; hymenium typically concave, discoid; basidia simple ..... *Cyphella*

Fructification consisting of only a fleshy hymenium on the surface of living leaves and shoots; basidia simple ..... *Exobasidium*

Fructification coriaceous or hard 1

1. Basidia globose or pyriform, longitudinally cruciately 4-septate or divided when mature; fructification erect, clavariform, more or less branched ..... *Tremellodendron*

[1] Cultural studies on the polymorphism of Hymenomycetes. Proc. Boston Soc. Nat. Hist. 33: 151–60. 1907.

1. Basidia cruciate as in *Tremellodendron;* fructification effuso-reflexed or cup-shaped with the margin free ..... *Hirneolina*
1. Basidia cruciate as in *Tremellodendron;* fructification always resupinate. *Sebacina*
1. Basidia simple but with such large sterigmata as to resemble longitudinally divided basidia[1] ..... *Tulasnella*
1. Basidia at first globose and simple, at length elongated and transversely septate, straight or curved, bearing sterigmata on the convex side; fructification resupinate ..... *Septobasidium*
1. Basidia simple, usually 4-spored 2
   2. Spores colored; fructification pileate ..... *Thelephora*
   2. Spores colored, rough-walled to echinulate; fructification resupinate ..... *Hypochnus*
   2. Spores ochraceous, ferruginous or fuscous, even; fructification resupinate .. *Coniophora*
   2. Spores white or rarely bright colored, even or rarely uneven 3
3. Setæ (brown, cylindric, rigid, even-walled bodies) present in the hymenium; fructifications range from pileate to resupinate ..... *Hymenochæte*
3. Cylindric teeth composed of many consolidated hyphæ protrude from the hymenium but are not covered by it. Our southern species was originally described as a *Hydnum* ..... *Mycobonia*
3. Neither setæ nor teeth present in the hymenium 4
   4. Fructification coriaceous, erect, clavariform; stem often hairy .. *Lachnocladium*
   4. Fructification cup-shaped, resupinate with free margin or simply resupinate; hymenium pulverulent; with some two or three of the following characters: (1) large white spores ranging from 14–34 x 12–20 μ; (2) much granular matter in the fructification; (3) prominent moniliform or branched paraphyses; (4) racemose organs in the hymenium which produce a crop of conidia before basidiospores develop ..... *Aleurodiscus*
   4. Fructification pileate ranging from infundibuliform and flabelliform to very narrowly reflexed forms; hymenium even. Some reflexed species may occur resupinate ..... *Stereum*
   4. Fructification like that of an urn-shaped *Stereum* but hard and stuffed. One tropical species ..... *Hypolyssus*
   4. Fructification like that of *Stereum* but with the hymenium hardened and with radiating branched ribs. Species tropical ..... *Cladoderris*
   4. Fructification always resupinate; structure not as in *Aleurodiscus* 5
5. Subhymenial tissue contains conspicuous brown stellate organs composed of several radiating arms ..... *Asterostroma*
5. Such brown stellate organs not present 6
   6. Cystidia present in hymenium, or in subhymenial tissue, or in both; structure may be compact or hypochnoid ..... *Peniophora*
   6. Cystidia not present; structure compact or hypochnoid ..... *Corticium*

[1] With regard to the nature of these bodies see H. O. Juel, Bihang till K. Sv. Vet.-Akad. Handl. 23[II]: Afd. III. 3–27. 1897.

II. HYMENO-LICHENS:

Fructification regularly containing green lichen gonidia. Species tropical.

Fructification pileate, coriaceous-membranaceous, with hymenium on the lower surface and somewhat waxy; gonidial layer composed of somewhat cubical masses of algal cells ..... *Cora*

Fructification like *Cora* in most respects but with the hymenium somewhat gelatinous and the gonidial layer composed of algal cells arranged in rows (cateniform) ..... *Rhipidonema*

THELEPHORA

Thelephora Ehrhart [Crypt. Exsic. No. 178. 1785] Fries, Syst. Myc. **1**: 428. 1821 (in part).–Persoon, Myc. Eur. **1**: 110. 1822 (in part).–Saccardo, Syll. Fung. **6**: 521. 1888 (in part).–Hennings, in Engl. &. Prantl, Nat. Pflanzenfam. (**1.** 1**): 125. 1898 (in part).

The type species of the genus is *Thelephora terrestris* Ehrh. ex Fries.

Fructifications pileate or clavate, coriaceous; hymenium continuous with the hymenophore and similar to it, inferior, or amphigenous in a few species, even or faintly ribbed or papillose; basidia simple, 4-spored; spores colored, typically muricate but even, or rough-walled in a few species.

As more broadly defined by Fries and the other authors cited, *Thelephora* has been heterogeneous, consisting chiefly of the natural and homogeneous group of species defined above but also of some pileate species with simple basidia and hyaline spores, transferred to *Stereum;* also of some species with globose, longitudinally septate basidia, transferred to *Tremellodendron,* if with erect fructifications, or to *Sebacina,* if resupinate; and also of some resupinate species having simple basidia, of which those with muricate and colored spores may be found in *Hypochnus,* those with colored and even spores, in *Coniophora,* and those with hyaline spores, in *Corticium* and *Peniophora.* It is probable that the species of Patouillard's section *Dendrocladium* of the genus *Lachnocladium* as understood by Patouillard [1] might be transferred to *Thelephora* with advantage both to *Thelephora* and *Lachnocladium,* but these species are not within the geographical limits of my work.

[1] Fragments Mycologiques (suite). Jour. de Bot. **3**:33–37. 1889.

KEY TO THE SPECIES

Erect species, usually with central stem and pileus divided into very narrow, branching, flattened or cylindric divisions; hymenium inferior or amphigenous 1

Erect species, usually with central stem and more or less infundibuliform, cup-shaped or flabelliform pileus, which may be radially split into lobes and divisions 2

Species of incrusting, effuso-reflexed, dimidiate, or applanate habit 5

1. 2–6 cm. high, much branched, glabrous, with fetid odor when growing, perhaps rarely odorless ..... *1. T. palmata*

1. 3–5 cm. high, much branched, minutely pubescent; stem villose, without fetid odor. Compare *T. multipartita* ..... *2. T. anthocephala*

1. Less than 2½ cm. high, branching at or below surface of ground, dusky drab except at base ..... *3. T. cæspitulans*

1. Less than 2 cm. high, very slender and fragile, cinereous. Known only from State of Washington ..... *4. T. scissilis*

1. Large species, highly branched, with body of spore of regular obovoid form. Known only from Central America ..... *5. T. angustata*

2. Hymenium dark colored, i. e., brown to fuscous 3

2. Hymenium light colored, i. e., pallid to gray 4

3. Small species, 1½–3 cm. high, upper surface usually drying pallid, usually deeply cleft or many-parted into narrow divisions; stem villose. *6. T. multipartita*

3. Small species, 6 mm.–2½ cm. high, infundibuliform or deeply divided into two or three triangular divisions, or flabelliform; stem villose. Closely related to *T. multipartita* ..... *7. T. regularis*

3. Fructification 1 cm. high, white; stem white, glabrous. Known only from Guadaloupe ..... *8. T. pusiola*

3. 1½–5 cm. high, larger species than the three preceding but with thinner pileus, fuscous purple (Rood's brown) throughout, often with the thin lobes imbricate like the petals of a carnation; stem villose ..... *9. T. caryophyllea*

3. 2–4 cm. high, somewhat tubular, hymenium vinaceous brown to drab; stem sulcate and pitted but not villose; spores 10–14 μ in diameter. Known only from Jamaica ..... *10. T. magnispora*

3. Large species, 2½–7 cm. in diameter, with upper surface pallid except at the center and with the hymenium dark ..... *13. T. vialis*

4. Small species, less than 2 cm. in height and in diameter, somewhat pallid to brick-red ..... *7. T. regularis*

4. Pileus with outer lobes forming a cup and with inner lobes distinct, crowded, erect, cinereo-fuscous. Known from Costa Rica and Brazil. *11. T. corbiformis*

4. Large species, 5–7 cm. broad, deeply infundibuliform, habit and color of *Craterellus cornucopioides*. Costa Rica and Jamaica ..... *12. T. cornucopioides*

5. Growing in applanate clusters, effuso-reflexed, or dimidiate 6

5. Always incrusting (*T. albido-brunnea* is sometimes incrusting) 8

6. Hymenium pale and colored like the pileus, cinnamon-buff; pileus spongy, more than 2 mm. thick; spores 8–10 x 6–8 μ ..... *14. T. albido-brunnea*

6. Hymenium and pileus yellowish, less than 2 mm. thick; spores 5–6 x 4 μ *15. T. lutosa*

6. Hymenium drab, becoming sage-green when crushed in 7 per cent potassium hydrate solution; pileus pinkish buff to cinnamon-brown with a broad pale margin ..... *16. T. cuticularis*

6. Hymenium ferruginous brown (Rood's brown) to fuscous 7

7. Pileus, when squamulose, with the fibers matted and agglutinated into appressed and wholly adnate squamules, margin dilated and whitish fimbriate becoming entire and concolorous ..... *17. T. intybacea*

7. Pileus not zonate, fibrous-squamulose and usually strigose, margin fibrous-fimbriate ..... *18. T. terrestris*

7. Pileus zonate, in other respects resembling the preceding species ..... *19. T. griseozonata*

8. Incrusting and ascending small plants, free branches somewhat terete but flattened towards the tips; spores umbrinous ..... *20. T. fimbriata*

8. Resupinate on leaves and twigs on the ground and sending up free, simple or branching trunks; spores fuscous. Known from Cuba only *21. T. perplexa*

8. Incrusting leaves, etc., on the ground and ascending as sessile flabelliform pilei which are dentate at the upper end or deeply divided, honey-yellow to tawny olivaceous throughout. Known from Cuba only .... *22. T. dentosa*

8. Typically effused, rising obliquely upward from the support as a cluster of small trunks which branch and terminate in spiculous tips. *23. T. spiculosa*

1. **Thelephora palmata** Scop. ex Fries, Syst. Myc. 1: 432. 1821. Plate 4. fig. 4.

Clavaria palmata Scop. Fl. Carn. 2: 483. 1760.–*Ramaria palmata* Holmsk. Fun. Dan. 1: 106. *pl.*—.1799. –*Merisma foetidum* Pers. Syn. Fung. 584. 1801.–*M. palmata* Pers. Myc. Eur. 1:113. 1822.–*Thelephora palmata americana* Peck, Rep. N. Y. State Mus. 53: 857. 1900.

Illustrations: Greville, Crypt. Fl. 1: *pl. 46.*–Holmskiold, Fun. Dan. 1: *pl.* of *Ramaria palmata.*–Krombholz, Abbild. und Beschr. *pl. 54. f. 24, 25.*–Nees, System *pl. 16. f. 151 B.*–Baillon, Dictionn. de Botan. 1: 737. *f. 7.*–Loudon, Encyc. of Plants *f. 16131.*–Winter, Crypt. Flora 1: 321.

Fructification coriaceous-soft, fuscous purple, drying cinnabar-brown or chestnut-brown, erect, very much branched, with very fetid odor; pileus with numerous somewhat fastigiate, palmate divisions which are even, flattened, dilated above, and with fimbriate and whitish tips; stem simple or soon branched; hymenium amphigenous; spores pale umbrinous under the microscope, sparingly echinulate, 10 x 7–8 μ.

Fructification of American specimens 2–6 cm. high, 1–3 cm. broad; stem 1–1½ cm. long, 1–2 mm. thick.

On moist ground in coniferous woods and also in grassy fields. Prince Edward Island to North Carolina and west to Illinois. June to October.

In the American collections of this species the divisions of the pileus are narrow and a short stem is present. The habit is so similar to that of *Thelephora anthocephala* that record of the fetid odor should always be made if observed. The ultimate branches may be more or less terete, leading to the variety *americana* Pk.

Specimens examined:

Exsiccati: Ell. & Ev., N. Am. Fungi, 1937.
Austria: *G. Bresadola.*[1]
Sweden: *L. Romell, 53.*
Canada: Rustico Bay, Prince Edward Island, *J. Macoun, 324.*
New Hampshire: Chocorua, *W. G. Farlow.*
? Vermont: no locality data for specimen in Frost Herb., Univ. of Vermont.
Connecticut: Manchester, *C. C. Hanmer, 1398.*
New York: Fischer's Island, *C. C. Hanmer, 196.*
New Jersey: *C. G. Lloyd, 4612.*
Pennsylvania: Bethlehem, *Schweinitz,* Syn. N. Am. Fungi, 612 (in Herb. Schw.); Trexlertown, *Dr. W. Herbst;* Kitanning, *D. R. Sumstine, 2;* West Chester, *B. M. Everhart,* Ell. & Ev., N. Am. Fungi, 1937.
Delaware: Newark, *H. S. Jackson.*
Dist. of Columbia: Washington, *O. F. Cook,* comm. by P. L. Ricker, 1, 3.
N. Carolina: Asheville, *H. C. Beardslee, 924.*
Ohio: Connecticut, *C. G. Lloyd, 4493.*
Illinois: Glencoe, *E. T. and S. A. Harper, 664, 665.*
Missouri: St. Louis, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 42560).

[1] With regard to the citation of specimens all except those of "Exsiccati" are in Burt Herb. which are cited without explicit reference to place in other herbaria. For example, the specimen cited, "Connecticut: Manchester, *C. C. Hanmer, 196,*" is in Burt Herb. The data given is that received with the specimen and may identify a duplicate in another herbarium. The location of all specimens in herbaria other than my own is designated by the name of the herbarium in parenthesis with the prefix "in." For example, the specimen cited, "Louisiana: St. Martinville, *A. B. Langlois* (in Lloyd Herb., 3000)," is in Lloyd Herb., but not in Burt Herb.

2. T. anthocephala Bull. ex Fries, Syst. Myc. 1: 433. 1821. Plate 4. fig. 1.

Clavaria anthocephala Bull. Herb. de la France 2: 197. *pl. 452. f. 1.* 1789.

Illustrations: Bulliard, *Ibid. pl. 452. f. 1.*–Sowerby, Col. Figs. Eng. Fun. *pl. 156.*–Berkeley, Outlines Brit. Fung. *pl. 17. f. 4.*–Dufour, Atlas des Champ. *pl. 70.*

Fructification coriaceous-soft, somewhat ferruginous, drying fawn-color or cinnamon-brown, inodorous; pileus pubescent, divided to the stem into flaps which are dilated upwards and fimbriate and whitish at the apex or divided into irregular, branched, erect branches; stem simple, equal, villose; hymenium even; spores pale umbrinous under the microscope, ranging from angular-tuberculate to tuberculate-echinulate, 8–10 x 7–8μ.

Fructifications 3–5 cm. high, 1–3 cm. broad; stem 1–1½ cm. long, 1–2 mm. thick.

On the ground in woods. Massachusetts and Ohio to Louisiana. June to August. Rare.

Our specimens of *T. anthocephala* and *T. palmata* have the same habit but may be separated, even when dried, by the fine pubescence of the pileus visible with a lens, and by the villose-tomentose stem of the former. The spores of *T. anthocephala* are further slightly paler and have shorter spines with broader bases than those of *T. palmata.*

Specimens examined:

Austria: *G. Bresadola.*

Massachusetts: Newton, *W. G. Farlow* (in Farlow Herb.).

New York: Van Cortlandt Park, N. Y. City, *L. O. Overholts* (in Overholts Herb., 688).

Pennsylvania: Kitanning, *D. R. Sumstine, 10;* Bethlehem, *Schweinitz* (in Herb. Schw.), the 614 of Syn. N. A. Fungi under the name *T. flabellaris.*

North Carolina: Asheville, *H. C. Beardslee, 0268.*

Louisiana: St. Martinville, *A. B. Langlois, unnumbered specimen,* and *1971*, and by the same collector (in Lloyd Herb., 3000).

Ohio: Norwood and Linwood, *C. G. Lloyd, 1472* and *02164* respectively.

Kentucky: *C. G. Lloyd, 1395.*

Missouri: St. Louis, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 42559).

3. T. caespitulans Schw. Trans. Am. Phil. Soc. N. S. 4: 166. 1831.[1]

Type: in Herb. Schweinitz.

Fructification erect, coriaceous, dusky drab to olive-brown below, paler above, very much branched, forming clusters $2\frac{1}{2}$ cm. high by $2\frac{1}{2}$ cm. broad; pileus with numerous divisions joined together into a solid base but assurgent above and pressed together closely, compressed, subcanaliculate, frequently obtuse and whitish at the apex; hymenium amphigenous; spores umbrinous under the microscope, sparingly tuberculate, 7–8 x 5–6μ.

On the ground in mixed woods, Vermont to South Carolina, and in dense coniferous woods, Washington. September. Rare.

This species is related to *T. palmata* but is more olivaceous, and it is probably inodorous,—at least no odor has been noted. The dimensions for the clusters given above, as stated by Schweinitz, are probably maximum dimensions, for the specimens recently collected have been rather smaller. My Vermont specimens were growing with the thick, solid base buried in sandy ground in a wood road; they have dried pallid except at the base and are slightly pubescent. The general habit of this species is somewhat suggested by a small cluster of *Tremellodendron pallidum* (Schw.) Atk.

Specimens examined:

Vermont: Lake Dunmore, *E. A. Burt.*

Pennsylvania: Bethlehem, *Schweinitz,* type (in Herb. Schw., Acad. Nat. Sci., Phila.).

South Carolina: Santee Canal, *Ravenel, 1660* (in Curtis Herb. under name *T. vialis*).

Washington: Chebalis, *C. J. Humphrey, 1287;* Bingen, *W. N. Suksdorf, 689.*

4. T. scissilis Burt, n. sp. Plate 4. fig. 8.

Type: in Burt Herb.

Fructifications gregarious, coriaceous, erect, clavariform, branched, longitudinally ridged by the bases of numerous,

[1] A figure will be given in Part II.

small, appressed, acicular branches, the larger of which are at the apex of the fructification and spread slightly outward in fan-shaped manner; stem glabrous, castaneous; hymenium amphigenous, on upper half of the fructification, avellaneo-cinereous; basidia simple, hyaline, 4-spored; spores pale umbrinous under the microscope, angular, 6–8 x 5–6μ.

Fructifications 1½–2 cm. high; spread of branches at the top 2–6 mm.; stem 7–10 mm. long, 1 mm. thick.

On the ground. Washington. January.

This species is very distinct by its slender erect habit, cinereous color, and only slightly spreading branches.

Specimens examined:

Washington: Bingen, Klickitat Co., *W. N. Suksdorf, 716*, type.

5. T. angustata Fries, (Nov. Symb. Myc. 92.) Actis R. Soc. Sc. Upsal. III. **1**: 108. 1851.

Type: in Herb. Fries.

Fructification erect, cinereo-fuscous, pliant, becoming rigid and somewhat woody; stem elongated, radicated, rugose, glabrous, compressed, irregularly divided at the upper end into unequal, fastigiate, compressed branches, which are clothed on the whole outer surface with the hymenium; hymenium amphigenous, subrugose, gray; basidia simple; spores umbrinous under the microscope, obovoid, apiculate at base, flattened on one side, echinulate, 14 x 7–9μ.

On decaying wood. Central America.

Substance, color, and hymenium exactly as in *T. cornucopioides*, but of the very different form of *Clavaria rugosa* and having highly branched forms; stem 5 cm. long; color fuliginous. The fructification is fleshy-pliant when fresh, but on drying hardens much more than species of *Stereum*.

Specimens examined:

Costa Rica: *Oersted* (in Herb. Fries), type.

6. T. multipartita Schw. in Fries, Elenchus Fung. **1**: 166. 1828. Plate 4. fig. 7a.

Type: in Herb. Schweinitz.

Fructifications gregarious, erect, coriaceous, fusco-cinereous, usually drying pallid; pileus infundibuliform, sometimes cleft

more or less deeply and unequally into a few lobes, sometimes divided to the stem and spreading so as to appear dimidiate, very often deeply divided and subdivided into many narrow and spreading divisions more or less dilated and whitish at the apex; stem erect or incurved, equal or tapering upward, sometimes branched above, drying walnut-brown or pallid, villose; hymenium inferior, glabrous, even, fawn-color or vinaceous drab; spores unbrinous under the microscope, tuberculate, 7–9 x 5–6μ.

Fructification $1\frac{1}{2}$–$3\frac{1}{2}$ cm. high, 1–3 cm. broad; stem 1–2 cm. long, 1–3 mm. thick.

On ground in groves of broad-leaved trees, especially under oak. New York and Pennsylvania to Illinois. July to September.

The upper surface of the pileus was originally described as glabrous, but it is minutely pubescent under a lens, or sometimes fibrillose. This species is very perplexing by its close relationship to *T. regularis*. The multipartite pileus is the only character which seems available to separate collections of the former from the latter species. If a given collection consists wholly of specimens with pileus many-parted and subdivided into narrow divisions, or if it contains some such specimens in addition to others with more regular infundibuliform pileus, I refer the collection to *T. multipartita*, as in the cases of the collections cited below from C. O. Smith and Dr. C. H. Peck respectively. As yet, I know of no characters by which to assort and separate into their respective species specimens mixed together of typical *T. regularis* and those specimens of *T. multipartita* which have the pileus infundibuliform or merely cleft more or less deeply and unequally into a few lobes. Therefore it is my opinion that *T. multipartita* is a variety of *T. regularis*, but the collections which have so far been submitted to me, have been composed of too few fructifications to assure me that this opinion is correct.

Specimens examined:

Exsiccati: Ell. & Ev., N. Am. Fungi, 2806, under the name *T. caryophyllea*.

New York: Bolton, *C. H. Peck, 3, 4, 5;* Ithaca, *C. O. Smith,* Cornell Univ. Herb., 13359, and *C. O. Smith and W. H. Long,* Cornell Univ. Herb., 7743.

New Jersey: Newfield, *J. B. Ellis,* Ell. & Ev., N. Am. Fungi, 2806.

Pennsylvania: on island in Lehigh River, *Schweinitz,* type (in Herb. Schw.); Bethlehem, *Schweinitz* (in Herb. Schw.), the *T. tuberosa* of Syn. N. Am. Fungi, 613; Trexlertown, *W. Herbst, 22, 36.*

Ohio: *A. P. Morgan,* Lloyd Herb., 2581, 2647; Oxford, *L. O. Overholts* (in Overholts Herb., 1685).

Illinois: River Forest, *E. T. and S. A. Harper, 666.*

7. T. regularis Schw. Schrift. d. Naturforsch. Gesell., Leipzig, 1: 105. 1822. Plate 4. figs. 6, 7b.

Thelephora Ravenelii Berk. Grevillea 1: 148. 1873.–*T. hiscens* Berk. & Rav. Grevillea 1: 148. 1873.

Type: in Herb. Schweinitz, Acad. Nat. Sci., Phila.

Pileus coriaceous, solitary, infundibuliform or divided to the stem into triangular divisions or flabelliform, fibrillose, drying pallid or tawny-olive, darker at center of the cup or at base of the divisions, margin lacerate; hymenium usually hair-brown, sometimes pallid; spores melleus to umbrinous under the microscope, angular-tuberculate, 6–7 x $4\frac{1}{2}$–5μ.

Fructification 6 mm.–$2\frac{1}{2}$ cm. high; pileus 5 mm.–$2\frac{1}{2}$ cm. broad; stem 3–15 mm. long, 1–$1\frac{1}{2}$ mm. thick.

In moss in wet places and on humus. Ontario to Alabama and westward to Kansas.

The differences in form of the pileus of *T. regularis* are well shown by the type in Herb. Schweinitz; this type consists of three fructifications, two of which are infundibuliform, the third and largest, flabelliform. The hymenium is sometimes merely pallid, as in the case of the specimen which is the *T. pannosa* of Schweinitz, Syn. N. Am. Fungi, No. 606, but is not *T. pannosa* Fr. The cotypes of *T. Ravenelii* and *T. hiscens* agree in all respects with the authentic specimen of *T. regularis* in Curtis Herb. Specimens of *T. regularis* which have the pileus infundibuliform and little cleft are suggestive of small specimens of *T. caryophyllea* but differ from the latter by the thicker pileus

and paler coloration of *T. multipartita* which is wholly lacking in the rufescent coloration of *T. caryophyllea*. There is a difference of form between specimens of these two species which is brought out well by the figures in pl. 4.

Specimens examined:

Canada: Shannonville, Ontario, *J. Macoun, 330.*

Maine: Portage, *L. W. Riddle, 4.*

New Hampshire: Chocorua, *W. G. Farlow* (in Farlow Herb.).

Massachusetts: near Boston, *Sprague, 246* (in Curtis Herb. under the name *T. anthocephala*); Newton, *W. G. Farlow* (in Farlow Herb.).

Pennsylvania: Bethlehem, *Schweinitz,* station cited by Schweinitz; also the specimen (in Herb. Schw.) under the name *T. pannosa* of Syn. N. Am. Fungi, No. 606; Trexlertown, *C. G. Lloyd;* Kitanning, *D. R. Sumstine.*

Delaware: Clayton, *H. S. Jackson.*

North Carolina: Salem, *Schweinitz,* type (in Herb. Schw.); *G. F. Atkinson,* Cornell Univ. Herb., 23254.

South Carolina: Greenville, *Ravenel, 1498,* type and cotype (in Kew Herb. and in Curtis Herb. respectively) of *T. Ravenelii* Berk.; Santee Canal, *Ravenel,* type and cotype (in Kew Herb. and in Curtis Herb. respectively) of *T. hiscens* Berk. & Rav.

Alabama: *Peters, 576 bis* (in Curtis Herb. under the name *T. anthocephala*).

Wisconsin: Madison, *W. Trelease* (in Farlow Herb.); Lake Geneva, *E. T. and S. A. Harper, 882,* and (in Harper Herb., 883).

Illinois: East St. Louis, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 42563).

Iowa: Johnson County, *T. J. Fitzpatrick, 39.*

Missouri: St. Louis, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 42564).

Kansas: Bourbon County, *A. O. Garrett, 80.*

8. T. pusiola Pat. in Duss, Champ. Guad. & Martinique 12. 1903.

Pileus with divisions triangular, white, hard, thin, entire or cut-lobed, glabrous, even or rugose, sometimes zonate, atten-

uated into a slender stem; stem colored like the pileus, glabrous, cylindric, woody; hymenium inferior, even, brown; basidia clavate, 25 x 10μ, four-spored; spores globose-angular, colorless or somewhat fuliginous, 6 μ in diameter; no cystidia.

Fructification 1 cm. high, divisions 5 mm. broad.

Solitary or in clusters on dead wood. Guadaloupe. Forest of Bains-Jaune, *Duss, 589.*

Var. *terrestris* Pat. *Ibid,* has the divisions of the pileus narrower, laciniate, divergent, rigid.

On the ground, Matouba, Guadaloupe, *Duss.*

I have seen no specimens of either this species or its variety, neither of which have been reported since their original discovery.

9. T. caryophyllea Schaeffer ex Fries, Syst. Myc. **1**: 430. 1821. Plate 4. fig. 9.

Elvella caryophyllea Schaeffer, Icon. Fung. **3**: 115. *pl. 325.* 1762–1774.–*Craterella ambigua* Pers. Obs. Myc. **1**: 36. *pl. 6. f. 8–10.* 1796.–*Thelephora caryophyllea* γ *ambigua* Pers. Myc. Eur. **1**: 112. 1822.

Illustrations: Schaeffer, Icon. Fung. *pl. 325.*–Persoon, Obs. Myc. **1**: *pl. 6. f. 8–10.*–Schnizlein, in Sturm, Deutsch. Flora **3**: fasc. 31. *pl. 6.*–Lanzi, Fungi di Roma *pl. 11. f. 4.*–Saunders and Smith, Myc. Ill. *pl. 41. f. 7–12.*–Smith, W. G. Brit. Basid. 399. *f. 96 a, b.*

Fructifications solitary or cespitose, coriaceous, fuscous purple but drying wood-brown; pileus infundibuliform, simple, or doubled by proliferous growth of smaller pilei from the disk of the principal pileus or of wedge-shaped lobes rising from its upper surface, upper surface radiately ridged or striate with masses of agglutinated fibers which are often dark colored, obscurely zonate when moist, margin incised; stem usually central, cylindric, villose, simple or branched; hymenium inferior, even, grayish olive to light yellowish olive; spores pale umbrinous, tuberculate, 7–8 x 6μ.

Fructification $1\frac{1}{2}$–5 cm. high, $1\frac{1}{2}$–5 cm. broad; stem 1 cm. long, 2–3 mm. thick.

On the ground under pines. Canada to South Carolina and west to Ohio, also in the Pacific states. August to November. Abundant locally.

T. caryophyllea may be distinguished from our other northern species which have a central stem and dark hymenium, by the thin lobes of the pileus which dry paler than the hymenium, and by the frequent occurrence of specimens with the pileus consisting of many lobes and pilei imbricately arranged in a manner suggestive of a double pink or carnation, as shown by Schaeffer's fig. 5, and Persoon's fig. 10 of the illustrations cited. Our specimens agree well with the figures of Schaeffer and Persoon—those of Persoon are especially good but unfortunately occur in a work which is very rare.

We find occasionally specimens which agree well with *T. radiata* (Holmsk.) Fr., but these specimens are connected so closely by intermediate forms—often in the same collection—with others which are undoubtedly *T. caryophyllea* that I refer them to the latter species.

Specimens examined:

Sweden: *K. Starback*, in Romell, Fun. Scand., 121.

Canada: *J. Macoun, 54* and *75* of 1903.

Quebec: Hull, *J. Macoun, 190.*

Ontario: London, *J. Dearness* (in Lloyd Herb.).

New Brunswick: Restigouche River, *T. F. Allen*, comm. by Dr. Farlow.

Maine: Orono, *L. W. Riddle, 9.*

New Hampshire: Shelburne, *W. G. Farlow.*

Vermont: Newfane, *C. D. Howe;* Middlebury, *E. A. Burt*, four collections.

Massachusetts: *Sprague, 47, Russell, 131*, and *D. Murray, 545* (all in Curtis Herb.); Worcester, *G. E. Francis, 105.*

Connecticut: East Hartford, *C. C. Hanmer, 1449;* Central Village, *J. L. Sheldon, 68*, comm. by New York Bot. Gard.

New York: Bolton, *C. H. Peck;* Ithaca, *G. F. Atkinson, 9993, 9994;* Saranac Lake, *E. A. Burt;* East Galway, *E. A. Burt.*

Pennsylvania: Bethlehem, *Schweinitz* (in Herb. Schw.), the 608 of Syn. N. Am. Fungi.

Dist. of Columbia: Zoölogical Park, *Coville and Cook, No. A*, comm. by P. L. Ricker.

North Carolina: *Schweinitz* (in Herb. Schw.).

Michigan: *C. G. Lloyd, 4547;* Sailor's Encampment, *E. T. and S. A. Harper, 439,* and Univ. of Wis. Herb., 2.
Ohio: *C. G. Lloyd, 1422, 2720;* Cincinnati, *A. P. Morgan,* Lloyd Herb., 2641, and (in Lloyd Herb., 1152); Loveland, *D. L. James* (in Herb. U. S. Dept. Ag.).
Kentucky: *C. G. Lloyd, 1152.*
Washington: Bingen, *W. N. Suksdorf, 717, 690.*
California: Jackson, *J. H. Barber,* comm. by W. A. Setchell; Stanford University, *C. F. Baker, 255,* distributed by Baker, Pacific Slope Fungi, 3743, under the name *T. radiata* (Holmsk.) Fr.

10. T. magnispora Burt, n. sp. Plate 4. fig. 5.

Type: in Burt Herb.

Fructifications solitary or gregarious, coriaceous, stipitate; pileus irregularly infundibuliform, somewhat tubular, with ascending recurved lobes, drying avellaneous, becoming fuscous at the center with age, fibrous torn becoming radiately striate, margin incised; stem equal, solid, drying hard, irregularly angled, sulcate and pitted, vinaceous brown to drab; hymenium inferior, even, vinaceous brown; basidia simple; spores pale cinnamon, subglobose, echinulate, 10–14 μ in diameter.

Fructification 2–4 cm. high; pileus 1–2 cm. in diameter; stem 7–12 mm. long, 2–5 mm. thick.

On mossy ground. Chester Vale, Jamaica. December.

In some of the specimens the pileus is decidedly eccentric through greater growth on one side than on the other, and it is not always lobed. The offensive odor of the dried specimens and the color of the hymenium are suggestive of *T. cuticularis.*

Specimens examined:
Jamaica: Chester Vale, *W. A. and Edna L. Murrill,* type, New York Bot. Gard., Fungi of Jamaica, 295.

11. T. corbiformis Fries, (Nov. Symb. Myc. 92.) Actis. R. Soc. Sc. Upsal. III. 1: 108. 1851.–Romell, Hymenomycetes Austro-Americani. Bihang till K. Sv. Vet.–Akad. Handl. **26**[16]: Afd. III. 44. 1901.

Type: in Herb. Fries.

Fructification sessile, rigid, cinereo-fuscous, with cespitose lobes of which the outer ascend and coalesce into a rounded

cupulate pileus here and there lacunose-pervious, and the inner are distinct, crowded, erect, narrow; hymenium inferior, uneven, whitish; basidia simple; spores slightly colored, becoming uneven, ovoid, 5–6 x 4–5 μ.

On the ground. Costa Rica and Brazil. January.

"In substance, texture, color, etc., this species agrees exactly with *Thel. cornucopioides* and *Thel. angustata* but in form it exhibits a type unique in the Hymenomycetes. The clusters are regularly rounded, very dense, divided all the way to the base into innumerable lobes, of which the interior are free and erect, the exterior regularly ascendant, broader, compressed, clothed underneath by the hymenium and grown together into a cup here and there lacunose-pervious, undulate-crisped at the apex and fimbriate."—Translation of the original comment on this species.

In 1899, I found the type in Herb. Fries to be cinereo-pallid with a slight fuscous tinge and with basidia and spores as stated above but many of the spores even. Romell describes the spores of his specimens from Brazil as "hyalinæ, laeves, ellips., 5–7 x 3–4 mmm.," and as agreeing with the type. I have reëxamined my sections from the type; the spores are certainly colored and many of them rough-walled.

Specimens examined:

Costa Rica: San José, *Oersted* (in Herb. Fries, Univ. Upsal.), type.

12. T. cornucopioides Fries, (Nov. Symb. Myc. 91.) Actis R. Soc. Sc. Upsal. III. **1**: 107. 1851.[1]

Type: not known to be in existence; not in Herb. Fries, at Upsala, nor in Kew Herb.

Pileus pliant becoming rigid, deeply infundibuliform, 5–7½ cm. broad, radiately rugose, glabrous, fuscous; stem solid, rather glabrous, pallid; hymenium inferior, somewhat rugose, gray.

On the ground. Near San José, Costa Rica.

This species bears so singular a resemblance to *Craterellus cornucopioides* that from pictures they are scarcely to be distinguished. The present species has the stem truly solid and the substance fleshy pliant when living, nearly stony-woody when dry; stem 5–7½ cm. long, 4–6 mm. thick, equal or attenu-

[1] A figure will be given in Part II.

ated at the base, compressed, rather glabrous, very tough, pallid; pileus membranaceous-cartilaginous, when dry quite rigid, radiately rugose, with the ridges elevated towards the undulate and at first fimbriate margin, not zonate after the manner of species of *Stereum;* hymenium inferior, hardened. Related to *Cladoderris.*

I refer to *T. cornucopioides* a collection made in Jamaica by Prof. F. S. Earle, in 1902, the specimens of which agree well with the original description, as translated above, except in size. They are 3–3½ cm. high and 2 cm. broad with stem about 1 cm. long by 2–4 mm. thick. The dried fructification is very hard and stony and softens so little with water that the edge of the razor is turned in sectioning. The spores are colorless and even at first and become slightly colored and angular, 9–10 x 6μ.

Specimens examined:

Jamaica: Castleton Gardens, F. S. Earle, New York Bot. Gard., Plants of Jamaica, 238.

13. T. vialis Schw. (Syn. N. Am. Fungi) Trans. Am. Phil. Soc. N. S. **4**: 165. 1834. Plate 5. fig. 15.

T. tephroleuca B. & C. Grevillea **1**:149. 1873.

Type: in Herb. Schweinitz.

Fructification coriaceous, dirty whitish or pallid, sometimes wood-brown at the center, upper surface usually radiately plicate or rough with masses of agglutinated fibers; pileus polymorphic, sometimes composed of ascending lobes or small pilei which arise from a common base and grow together above to form a broad cup, or sometimes with the whole interior of the cup filled with small pilei and lobes many of which arise proliferously from the upper surface of the outer lobes; stem central when present; hymenium inferior, rugose, somewhat papillose, yellowish pallid becoming avellaneous or somewhat fuscous; spores olive-buff under the microscope, bluntly angular (i. e., tips of the angles obtuse), 4½–7 x 4½–5μ.

Fructification 2½–5 or 6 cm. high, 2½–7 cm. broad.

On ground in frondose woods. Vermont to South Carolina and west to Illinois. September.

This is a fine, large species well marked by the dirty whitish or yellowish, fibrillose upper surface of the pileus, thick substance of the same color unless the specimen is old, and the brown,

slightly wrinkled hymenium. As in the otherwise very different *T. caryophyllea*, large specimens sometimes resemble a double flower from the great number of small pileoli and lobes present in the center. Schweinitz described the species as sometimes having dimidiate pilei, but I have seen no such specimens. My collection assumed a disagreeable odor in drying but no such odor has been noted by others.

Specimens examined:

Exsiccati: Ell. & Ev., N. Am. Fungi, 1110, and Fun. Col., 1593, in both under the name *T. caespitulans*.

Vermont: Lake Dunmore, *E. A. Burt.*

New Jersey: Newfield, *J. B. Ellis* (in Mo. Bot. Gard. Herb., 5155), also in the exsiccati cited.

Pennsylvania: Bethlehem, *Schweinitz,* type (in Herb. Schw.); *Michener, 1504* (in Curtis Herb. and in Kew Herb.), the cotype and type respectively of *T. tephroleuca;* Trexlertown, *W. Herbst, 43, C. G. Lloyd and W. Herbst, 2866, 3088* (both in Lloyd Herb.); *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 42561).

Dist. of Columbia: Washington, *F. J. Braendle,* comm. by C. H. Peck.

North Carolina: *G. F. Atkinson* (in Cornell Univ. Herb., 23253); Asheville, *H. C. Beardslee;* Schweinitz cited North Carolina as a station.

South Carolina: Caesar's Head, *Ravenel,* one of the types (in Curtis Herb. and Kew Herb.) of *T. tephroleuca.*

Ohio: *C. G. Lloyd, 4000.*

Illinois: Glen Ellen, *E. T. and S. A. Harper, 669.*

14. T. albido-brunnea Schw. Trans. Am. Phil. Soc. N. S. **4**: 166. 1834. Plate 5. fig. 13.

Stereum Micheneri B. & C. Grevillea **1**: 162. 1873 (in part).–*Stereum spongiosum* Massee, Jour. Linn. Soc. Bot. **27**: 172. 1889.–*Thelephora odorifera* Peck, Rep. N. Y. State Mus. **44**: 132 (22). 1891.

Type: in Herb. Schweinitz.

Pileus sessile or with very short stem, coriaceous, spongy when dry, uniformly cinnamon-buff or with the older portions chestnut-brown, sometimes assuming mesopod form when encircling small twigs or shrubs, sometimes effuso-reflexed, usually dimidi-

ate and somewhat imbricated, fibrous-tomentose, margin thick and entire; substance concolorous with the upper surface, spongy, more than 2 mm. thick, with hyphae $4\frac{1}{2}$–5μ in diameter; hymenium inferior, even, not polished, cinnamon-buff; basidia simple; spores deep olive-buff under the microscope, echinulate, 8–10 x 6–8μ.

Pileus 2–4 cm. in diameter when circular, or 1–$2\frac{1}{2}$ cm. long, 2–4 cm. broad, often 1 cm. thick at base when dimidiate.

Running up and encircling twigs on the ground and against the base of shrubs. Canada to Louisiana and west to Wisconsin. August.

Peck describes the odor as quite fragrant at first but states that it is lost after a few weeks; I did not notice any especial odor for my collection. *T. albido-brunnea* may be distinguished from our other dimidiate and reflexed species of *Thelephora* by its even and pale hymenium and thick spongy pileus. Schweinitz confused one collection of this species with *T. biennis* Fr., from the specimen of which in the Fries Herbarium, at Upsala, it is clearly distinct. The types of *Stereum spongiosum* Massee, viz., *Curtis, 3582,* and *Ravenel, 1732,* in Kew Herbarium, have colored echinulate spores 8–10 x 6–7μ, although described by Massee as "ellipsoideæ 6–7 x 4μ" without mention of color and projections of the wall. The type of *Thelephora odorifera* Peck, in Coll. N. Y. State, is somewhat bleached or faded but quite typical.

Specimens examined:

Exsiccati: Ravenel, Fun. Car. IV, 12, the type distribution of *T. Micheneri* B. & C.; Ell. & Ev., N. Am. Fungi, 1599, and Fun. Col., 1209, under the name *T. Micheneri* in both.

Canada: Toronto, *J. Dearness* (in Lloyd Herb.).

Vermont: Lake Dunmore, *E. A. Burt.*

New York: Selkirk, *C. H. Peck* (in Coll. N. Y. State), the type of *T. odorifera* Pk.; Alcove, *C. L. Shear, 1010, 1163, 1184;* Jamesville, *L. M. Underwood.*

Pennsylvania: Bethlehem, *Schweinitz* (in Herb. Schw.), the type, and also the Nos. 627 and 625 of Syn. N. Am. Fungi under the names respectively of *T. biennis* and *T. laciniata; Michener* (in Curtis Herb., 3582, and also in Kew Herb., same number), type of *Stereum spongiosum* Massee; Trexlertown, *W. Herbst, 18,* and (in Lloyd Herb., 3052).

North Carolina: Blowing Rock, *G. F. Atkinson, 4322.*
South Carolina: *Ravenel, 790* (in Curtis Herb. and in Kew Herb.), under the name *Thelephora biennis;* Santee Canal, *Ravenel, 1732* (in Curtis Herb. and in Kew Herb.), type of *Stereum spongiosum* Massee.
Louisiana: Bogalusa, *C. J. Humphrey, 466.*
Ohio: Cincinnati, *A. P. Morgan,* Lloyd Herb., 2627.
Michigan: Saugatuck, *E. A. and S. A. Harper, 654.*
Wisconsin: Milwaukee Co., comm. by Mrs. F. W. Patterson.

15. T. lutosa Schw. Trans. Am. Phil. Soc. N. S. 4: 166. 1834.[1]
Type: in Herb. Schweinitz.

Pilei cespitose, densely imbricated, at first somewhat fleshy but at length hard, undulate-plicate, yellowish, almost subtomentose with pulverulence, somewhat horizontally attenuated behind, margin sublobate, at length inflexed; pileus less than 2 mm. thick, with hyphae 3μ in diameter; hymenium becoming yellowish, even; spores olive-buff under the microscope, angular, 5–6 x 3$\frac{1}{2}$–4μ.

Cluster about 1$\frac{1}{2}$ cm. high and broad.

On the ground in roads and in woods. North Carolina.

The type is distinct from *T. albido-brunnea,* having thinner pileus, finer hyphae, and smaller and paler spores. The pilei were crowded together into a small buff-colored cluster about 1$\frac{1}{2}$ cm. high and broad, somewhat as in *Tremellodendron pallidum* (Schw.); I failed to find stems at their bases.

Specimens examined:
North Carolina: Salem, *Schweinitz* (in Herb. Schw.), type.

16. T. cuticularis Berk. Hooker's Lond. Jour. Bot. **6**: 324. 1847. Republished in Lea, Catalogue of Plants in Vicinity of Cincinnati 66. *d.* 1849. Plate 5. fig. 14.

Type: in Kew Herb., and a portion of it from Berkeley in Curtis Herb.

Pilei coriaceous-soft, effuso-reflexed or dimidiate, imbricate, sometimes laterally confluent, drying pinkish buff to cinnamon-brown, with a broad, pale margin, surface radiately rugose, soft, silky fibrillose; substance of the same color as pileus; hymenium inferior, concave, even, drab to brownish drab; spores umbrinous under the microscope, flattened on one side or somewhat kidney-shaped, not angular, echinulate, 8–9 x 6–7μ.

[1] A figure will be given in Part II.

Pileus 1–1½ cm. long, 2–4 cm. broad, 1 mm. thick.

On mossy bark at the base of trees and on fallen twigs in groves. Vermont to Texas and west to Missouri. June to August.

In his description Berkeley noted that the odor of this species is strong and unpleasant; my specimens retained such an odor for several years but I did not notice it before they were dried. *T. cuticularis* may be distinguished from our other species by its drab hymenium, portions of which become sage-green when crushed under a cover glass in a 7 per cent solution of potassium hydrate, and by its spores, which are not at all angular or irregular as regards the main body of the spore, but ovoid and flattened on one side or slightly kidney-shaped and sparingly studded with slender spines.

Specimens examined:

Vermont: Middlebury, *E. A. Burt.*
Rhode Island: *Olney, 1851* (in Kew Herb. and in Curtis Herb.).
Pennsylvania: Bethlehem, *Schweinitz* (in Herb. Schw.), the Nos. 628 and 629 of Syn. N. Am. Fungi, under the names respectively of *T. fuscocinerea,* and *T. gausapata;* Kitanning, *D. R. Sumstine, 1.*
Delaware: Newark, *H. S. Jackson.*
North Carolina: Asheville, *H. C. Beardslee, 03195.*
Florida: *Mrs. Sams,* comm. by C. G. Lloyd.
Texas: *W. H. Long, Jr., 351, 387* (in Cornell Univ. Herb.).
Ohio: Waynesville, *T. G. Lea* (in Kew Herb.), type; Preston, *A.P. and L. V. Morgan,* comm. by C. G. Lloyd, also *C. G. Lloyd,* specimen dated July 26, 1896; Cincinnati, *C. G. Lloyd, 4492.*
Wisconsin: Blue Mounds, *E. T. and S. A. Harper, 861.*
Missouri: Columbia, *B. M. Duggar, 289.*

17. **T. intybacea** Pers. ex Fries, Syst. Myc. 1: 431. 1821.
Plate 5. fig. 11.

T. intybacea Pers. Syn. Fung. 567. 1801–1807; Myc. Eur. 1: 110. 1822.

Illustrations: Bulliard, Champ. de la France *pl. 278.*–Bigeard et Guillemin, Champ. Super. France 436. *pl. 44. f. 1.*

Fructifications cespitose, soft, whitish, then rufous-ferruginous, drying chestnut-brown to Rood's brown, with stems

somewhat lateral and growing into one another; pilei imbricated, fibrous, usually with the fibers matted and agglutinated into appressed and wholly adnate squamules, margin dilated and whitish-fimbriate at first, at length becoming entire and colored like the rest of the pileus; hymenium inferior, concolorous with the upper surface, papillose; spores concolorous with hymenium, snuff-brown under the microscope, angular-tuberculate, 7–9 x 6–8μ.

Clusters often 5–8 cm. in diameter; individual pileus 2–3 cm. long, 2–4 cm. broad, 1 mm. thick.

On the ground in pine woods, growing up from the layer of fallen leaves. Ontario to North Carolina and westward to Ohio and Michigan. August to October.

The clusters are sometimes central but more often with the pilei lateral and triangular; sometimes the mass ascends small sticks and then extends out from this support in reflexed forms; the upper surface is usually uneven and dries somewhat depressed between the adnate squamules. This species is distinguished from ferruginous specimens of *T. terrestris* by the thicker and entire margin of the pileus and by the absence of free squamules.

Specimens examined:

Exsiccati: Ell. & Ev., Fun. Col., 1410.
Austria: *G. Bresadola.*
Ontario: Toronto, *J. Dearness*, comm. by C. G. Lloyd; Harraby, Lake Rosseau, *E. T. and S. A. Harper, 682.*
Maine: Portage, *L. W. Riddle, 3.*
New Hampshire: Shelburne, *W. G. Farlow.*
Vermont: Middlebury, Sudbury, Grand View Mt., *E. A. Burt.*
Massachusetts: *A. P. D. Piguet*, comm. by Dr. Farlow; Natick, *G. E. Morris, No. E.*
Connecticut: East Hartford, *C. C. Hanmer, 1434.*
New York: Alcove, *C. L. Shear, 1009;* East Galway, *E. A. Burt;* Ithaca, *G. F. Atkinson,* Cornell Univ. Herb., 3050, 19652.
Dist. of Columbia: Takoma Park, *C. L. Shear, 799, 796;* Washington, *O. F. Cook, 4,* comm. by P. L. Ricker.
North Carolina: Asheville, *H. C. Beardslee, 0341.*
Ohio: *A. P. Morgan* (in Lloyd Herb.).
Michigan: *C. G. Lloyd, 4546;* Lawton, *L. A. Hawkins;* Sailor's Encampment, *Allen and Stuntz, 1,* Univ. of Wis. Herb.

18. T. terrestris Ehrh. ex Fries, Syst. Myc. 1: 431. 1822.
Plate 5. fig. 10.

T. terrestris Ehrh. Crypt. Exsicc. No. 178. 1785.–Persoon, Syn. Fung. 566. 1801; Myc. Eur. 1: 113. 1822.–*Stereum laciniatum* Pers. Obs. Myc. 1: 36. 1796.–*Thelephora laciniata* Pers. Syn. Fung. 567. 1801.–*T. caryophyllea β laciniata* Pers. Myc. Eur. 1: 112. 1822.–*T. laciniata* Fries, Syst. Myc. 1: 431. 1821.

Illustrations: Batsch, Elenchus Fung. *pl. 24. f. 121.*–Nees, System der Pilze *pl. 34. f. 251.*–Bolton, Hist. Fung. *pl. 173.*–Sowerby, Col. Fig. of Eng. Fungi *pl. 213.*–Cooke, Handbook 1: 310.–Stevenson, Brit. Hym. 2: 261.–Smith, Brit. Basid. 399. *f. 96 C-E.*

Fructifications dark fuscous to fawn-color, coriaceous-soft, cespitose, obconic, with a short stem-like base, or dimidiate and sessile, or incrusting and effuso-reflexed; pileoli more or less imbricated, sometimes laterally confluent, fibrous-squamulose and usually strigose, thin, margin fibrous-fimbriate and laciniate; hymenium inferior, papillose, fuscous to fawn-color; spores pale fuscous, irregular, angular, sometimes slightly tuberculate, 6–9 x 6μ.

Clusters 5–8 cm. in diameter, with single pileolus about 3 cm. long and broad; obconic pileus 2–3 cm. in diameter; dimidiate pileolus 1½–2 cm. long, 2–3 cm. broad, about 1 mm. thick.

On sandy ground in bare fields and at base of trunks and from fallen twigs and leaves in pine woods. Canada to South Carolina, and in Michigan, Jamaica, and Alaska. July to December.

My observations of this species acquired from specimens received and from seeing it growing abundantly near Middle Grove, N. Y., seem to show that the medium from which this fungus derives its food produces an interesting effect on the fructification. Growing from bare, sandy ground the fructifications are dark fuscous in color, and may be flattened clusters of imbricated pileoli, or of the obconic-pileus type composed of ascending pileoli confluent laterally, or dimidiate, sessile pileoli. When growing on abundant woody matter, as is the case in the specimen in Sowerby's illustration already cited, the fructification assumes a redder color and replaces its dimidiate, sessile pileus on earth by a reflexed one on the wood. With regard to

other forms of the clusters and pileoli, the covering of the upper surface, and the spore characters there is no difference between those fructifications produced without woody food and those having it. There is no sharp color separation between these color extremes.

Specimens growing on the ground usually have a short stem-like base, while those growing on wood are reflexed; the same collection may show both these conditions, as, for example, that from Skagway, Alaska, if some of the fructifications start from sticks and others directly from the ground. Persoon regarded the stem in *T. terrestris* as the chief character separating that species from his *T. laciniata*, as may be seen from his own descriptions contrasting the two in his 'Synopsis Fungorum,' pp. 566 and 567, as follows:

"3. THEL. TERRESTRIS: subimbricata obscure fusca, pileo applanato fibroso-strigoso."

"Hab. in arenosis ad terram. *Stipes* breuis, lateralis omnino adest. Substantia submollis, non ita coriacea sicca, vti in ceteris speciebus."

"4. THEL. LACINIATA: imbricata obscure fusca, pileo tenui laciniato crispo subtus papillis congestis scabro."

"Hab. ad radices truncorum. Cespitem difformem efformat, 2 vnc. lata, tenuis. *Stip.* vix adest distinctus."

These descriptions supplement each other as a description for one species; each has special application to fructifications growing side by side under such conditions as to show that they are from a common mycelium. Persoon never claimed that his species differed from *T. terrestris* in color. Fries gave a different description of *T. laciniata* in his works cited—to the injury of *T. intybacea*—, but the characters he gives are not satisfactory. European mycologists with a wide knowledge of the *Thelephoraceæ* as they grow are unable to distinguish these two species. In letters to me, Bresadola regards *T. laciniata* as a synonym of *T. terrestris;* and Romell does not know *T. terrestris* if it is distinct from *T. laciniata.*

Specimens examined:

Exsiccati: Ellis, N. Am. Fungi, 511; Ell. & Ev., N. Am. Fungi, 2732, under the name *T. intybacea.*

Austria: *G. Bresadola.*

Sweden: *G. Romell, 52, 55, 56, 57.*

Newfoundland: *A. C. Waghorne, 276* (in Mo. Bot. Gard. Herb.).

Quebec: Gaspe, *J. Macoun, 229.*
Ontario: Ottawa and Belleville, *J. Macoun.*
Maine: Wells, *J. Blake,* comm. by P. L. Ricker.
New Hampshire: Chocorua, *W. G. Farlow.*
Massachusetts: Magnolia and Woods Hole, *W. G. Farlow;* Ipswich, *G. E. Morris, No. F.*
Connecticut: South Windsor, East Hartford, and Rockville, *C. C. Hanmer, 1227–29, 944, 1057.*
New York: East Galway and Middle Grove, *E. A. Burt,* three collections from the latter station; Ithaca, *G. F. Atkinson,* Cornell Univ. Herb., 22976.
New Jersey: Belleplain, *C. L. Shear, 1246;* Newfield, *J. B. Ellis,* Ellis, N. Am. Fungi, 511.
Pennsylvania: *Schweinitz* (in Herb. Schw.), the 624 of Syn. N. Am. Fungi.
North Carolina: Asheville, *H. C. Beardslee, 02280;* Salem, *Schweinitz* (in Herb. Schw.), the 624 of Syn. N. Am. Fungi.
Alabama: Tuskegee, *Beaumont, 199* (in Curtis Herb.).
South Carolina: Society Hill, *M. A. Curtis, 2693* (in Curtis Herb.).
Michigan: Agricultural College, *G. H. Hicks,* Ell. & Ev., Fun. Col., 2732.
Alaska: Skagway, *J. Macoun, 47; Evans, 410* (in Mo. Bot. Gard. Herb.).
Jamaica: Cinchona, *W. A. and E. L. Murrill,* New York Bot. Gard., Fun. of Jamaica, 451.

19. T. griseozonata Cooke, Grevillea **19:** 104. 1891.
Plate 5. fig. 12.

Type: in Ravenel, Fun. Amer., 444.

Fructifications cespitose, coriaceous-soft; pileoli extended into a short sublateral stem, imbricate, applanate, silky-strigose, zonate with alternating cervine (Rood's brown) and light buff zones, margin subfimbriate; hymenium inferior, castaneous when fresh, drying Rood's brown, rugose, somewhat papillose; spores pale fuscous, angular, 6–9 x 6–7μ.

Cluster 3–6 cm. in diameter; obconic pileus and single pileolus each 2–3 cm. in diameter.

On sandy ground in pine woods. New Jersey to Louisiana. August to November.

This species is closely related to *T. terrestris* and has the same habitat, habit of growth, and spore characters, but is distinguished from that species by its zonate pileus. The fructifications usually occur in flattened clusters with spreading pileoli; sometimes the individual pileoli acquire an infundibuliform appearance by the growing together for part of their length of opposite edges of individual pileoli; sometimes a small obconic pileus occurs composed of two or more pileoli with adjacent edges confluent. In the collection cited below from Mississippi, small lobes are present in the cavity of the cup, as in *T. vialis* and *T. caryophyllea*.

Specimens examined:

Exsiccati: Ravenel, Fungi Am., 444, type distribution; Ravenel, Fun. Car. II, 28, under the name *T. caryophyllea;* Ellis, N. Am. Fungi, 714; Ell. & Ev., Fun. Col., 1305.

New Jersey: Newfield, *J. B. Ellis,* in his exsiccati cited.

South Carolina: Aiken, *H. W. Ravenel,* Fungi Am., 444, type collection.

Alabama: Auburn, *C. F. Baker,* Lloyd Herb., 3462.

Mississippi: Biloxi, *Mrs. E. S. Earle, 32.*

Louisiana: St. Martinville, *A. B. Langlois, by.*

20. T. fimbriata Schw. ex Schweinitz, Trans. Am. Phil. Soc. N. S. **4**: 166. 1834. Plate 4. fig. 3.

Merisma fimbriatum Schw. (Syn. Fung. Car., No. 1067) Schrift. d. Naturforsch. Gesell., Leipzig, **1**: 110. 1822.–*Thelephora scoparia* Peck, Rep. N. Y. State Mus. **42**: 123 (27). *pl. 2. f. 20, 21.* 1889.

Illustrations: Peck, Rep. N. Y. State Mus. **42**: *pl. 2. f. 20, 21.*

Type: in Herb. Schweinitz.

Fructification coriaceous-soft, incrusting and ascending small plants (mosses, etc.), here and there emitting fascicles of branches united below, subterete, acuminate or fimbriately incised, at first pale or whitish, soon ferruginous brown, drying Rood's brown; hymenium even, pruinose-pubescent; spores umbrinous, tuberculate, 7–11 x 6–9 μ.

Incrusting and ascending upward 1–3 cm.; free branches 5–10 mm. long, 1 mm. thick, sweep of fascicle about 5–10 mm.

In moist places. New York to South Carolina, and west to Illinois. July and August.

The type is an incrusting specimen, covering as its main axis a small twig in one specimen and a moss in the other, and sending out a few lateral branches which are flattened towards the free ends and subfimbriate; main trunk is cylindric, latericius (of 'Chromotaxia'), ends of branches paler; spores umbrinous under the microscope, tuberculate, 7–8 x 6 μ. Schweinitz described the species as becoming hard and cartilaginous, but this is an error probably due to the foreign matter surrounded by the main trunk. Several other specimens are present in his herbarium under various names.

Specimens examined:

Exsiccati: Ellis, N. Am. Fungi, 512, under the name *T. cristata.*

Massachusetts: Weston, *A. B. Seymour, T 1* (in Mo. Bot. Gard. Herb., 45573).

New York: Bethlehem and Selkirk, *C. H. Peck* (in Coll. N. Y. State), type of *T. scoparia;* Syracuse, from Herb. Cornell Univ., 19474.

New Jersey: Newfield, *J. B. Ellis,* N. Am. Fungi, 512.

Pennsylvania: Bethlehem, *Schweinitz* (in Herb. Schw.), the 615 of Syn. N. Am. Fungi, under the name *T. stabularis.*

North Carolina: Salem, *Schweinitz* (in Herb. Schw.), type, and also the 1063 of Syn. Fung. Car., under the name *Merisma fuscescens.*

Indiana: Millers, *E. T. and S. A. Harper, 670.*

Illinois: Havana, *H. C. Beardslee;* Riverside, *E. T. and S. A. Harper, 668.*

21. T. perplexa Burt, n. sp.[1]

Type: in Curtis Herb.

Fructification incrusting, coriaceous, consisting of a resupinate membrane from the central portion of which arise cylindric trunks either simple or digitately branched; resupinate portion spongy, firm, separable, fuscous at the center, margin thin, determinate, pinkish buff; ascending portions spongy, firm,

[1] A figure will be given in Part II.

fuscous, simple and tapering upward or soon branching and terminating in paler either subulate tips or somewhat flattened ends; spores fuscous, subglobose, echinulate, 8–10 x 8–9μ.

The resupinate membrane may be 3 cm. in diameter; ascending portion of fructification 2–3 cm. high, $1\frac{1}{2}$–2 mm. thick.

On decaying leaves and sticks on the ground. Cuba.

Berkeley & Curtis based their description of *Thelephora dentosa* on two collections made in Cuba by C. Wright in different years; these collections are different specifically. The original description applies chiefly to the earlier collection, made in 1857, which is unnumbered. I take my type of *T. perplexa* from the later collection, *C. Wright, 238*.

Specimens examined:

Exsiccati: Fungi Cubenses Wrightiani, *C. Wright, 238*, under the name *Thelephora dentosa* B. & C.

Cuba: *C. Wright, 238*, type (in Curtis Herb.).

22. T. dentosa Berk. & Curtis emend Burt.[1]

T. dentosa B. & C. (Fungi Cubenses) Jour. Linn. Soc. Bot. **10**: 329. 1867.

Type: type and cotype in Kew Herb. and Curtis Herb. respectively.

Fructification coriaceous-soft, incrusting leaves and small twigs on the ground and ascending as free, sessile, dilated, triangular, flabelliform pilei which are dentate at the upper end or deeply divided into a few finger-shaped divisions, honey-yellow to tawny olivaceous throughout, minutely hairy under a lens; spores honey-yellow, globose to ovoid, weakly echinulate, 6–10 x 6–8μ.

Pileus 1 cm. high, 5 mm.–1 cm. broad.

On rotten vegetation. Cuba. June.

As already stated in connection with *T. perplexa*, Berkeley & Curtis cited for types of their *T. dentosa* specimens from two collections made in Cuba by C. Wright. These collections were made with an interval of several years between the collections, which differ specifically. As noted by Berkeley & Curtis, their description applies better to the earlier collection, to which I now

[1] A figure will be given in Part II.

restrict their species. This earlier collection was distributed by C. Wright, unnumbered, under the name *Thelephora dentosa* B. & C. before the publication of the description of this species, and the cotype in Curtis Herb. is unnumbered also. By what was apparently a slip of the pen, Berkeley cited this type as *C. Wright, 507*. By the kindness of Dr. Farlow I have been permitted to examine the manuscript records which show that Wright collected only one No. 507, which was determined by Berkeley as *Xylaria obovata* Berk. and is cited under this species by Berk. & Curtis, Jour. Linn. Soc. Bot. **10:** 380. 1867. I find in Curtis Herb. such a specimen labelled *Xylaria obovata* Berk., Cuba, *C. Wright, 507*. I conclude that the type and cotype of *T. dentosa* B. & C., first cited in their description, are from the collection distributed by C. Wright, unnumbered, under the name *Thelephora dentosa* B. & C.

Specimens examined:

Exsiccati: Plantae Cubenses Wrightianae, unnumbered, under the name *Thelephora dentosa* B. & C.

Cuba: *C. Wright*, cotype (in Curtis Herb.).

23. T. spiculosa Fries, Syst. Myc. **1:** 434. 1821; Epicr. Syst. Myc. 539. 1836–38. Plate 4. fig. 2.

Illustrations: Persoon, Syn. Fung. *pl. 3. f. 16.*

Type: an authentic specimen from Fries, in Kew Herb.

Fructifications cespitose, from byssoid becoming fleshy, variable by incrusting habit, pale buff at first, main portions becoming purplish-fuscous (Rood's brown) with age, ramose-spiculous, tips penicillate and whitish; spores umbrinous under the microscope, irregular, echinulate, 8–9 x 6–7 μ.

Clusters 1–2 cm. high, 2–4 cm. in diameter, single fructification 1–2 cm. high, about 1 mm. in diameter, with branches spreading 4–6 mm.

On leaves on ground in moist groves. Ohio to Wisconsin. July. Rare.

The best specimens which I have seen have main trunks of the fructifications running side by side over partially decayed beech leaves and confluent into an effused mass. These trunks ascend obliquely from the leaves to a height of 1–2 cm., branch sparingly, and terminate in spiculous tips. The fructification

must be inconspicuous in the woods since the general color of the mass is the same as that of the leaves on which it is effused, although the main trunks may be darker.

Specimens examined:

Exsiccati: Kunze, Fun. Sel. Exsic., 560.
Sweden: specimen from Fries (in Kew Herb.).
Austria: *G. Bresadola.*
Ohio: Preston, *C. G. Lloyd.*
Michigan: Glen Lake, *C. G. Lloyd, 02471.*
Wisconsin: Lake Geneva, *E. T. and S. A. Harper, 883.*

(To be continued.)

Explanation of Plate

PLATE 4

All figures of plates 4 and 5 have been reproduced natural size from photographs of dried herbarium specimens of species of *Thelephora.*

Fig. 1. *Thelephora anthocephala.* From specimen collected at Linwood, Ohio, by C. G. Lloyd, No. 02164.

Fig. 2. *T. spiculosa.* *a*, from specimen on leaves of *Fagus* collected in Europe by Bresadola, which I compared with the specimen from Fries in Kew Herbarium; *b*, from specimen collected at Glen Lake, Mich., by C. G. Lloyd, No. 02471.

Fig. 3. *T. fimbriata.* From specimen incrusting living strawberry (*Fragaria*) plant, collected at Riverside, Ill., by E. T. and S. A. Harper, No. 668.

Fig. 4. *T. palmata.* From specimen from New Jersey, from C. G. Lloyd, No. 4612.

Fig. 5. *T. magnispora.* From type specimens collected at Chester Vale, Jamaica, by W. A. and Edna L. Murrill, No. 295. *a* shows upper surface and side of pileus, and *b*, the hymenium.

Fig. 6. *T. regularis.* From a sketch of the type in Herb. Schweinitz.

Fig. 7 *a. T. multipartita.* From specimens collected at Trexlertown, Pa., by Dr. W. Herbst.

Fig. 7 *b. T. regularis.* From specimens collected at Clayton, Del., by H. S. Jackson.

Fig. 8. *T. scissilis.* From type specimens collected at Bingen, Wash., by W. N. Suksdorf, No. 716.

Fig. 9. *T. caryophyllea.* From specimens collected in Michigan, by C. G. Lloyd, No. 4547.

BURT — THELEPHORACEAE OF NORTH AMERICA

1. THELEPHORA ANTHOCEPHALA.—2. T. SPICULOSA.—3. T. FIMBRIATA.—4. T. PALMATA.—5. T. MAGNISPORA.—6 AND 7 b. T. REGULARIS.—7 a. T. MULTIPARTITA.—8. T. SCISSILIS.—9. T. CARYOPHYLLEA.

COCKAYNE, BOSTON.

Explanation of Plate

PLATE 5.

Fig. 10. *T. terrestris.* From specimens collected on ground in open fields at Middle Grove, N. Y. *a* shows the fibrose-strigose upper surface and fimbriate margin of the pileus, and *b*, the hymenium of lower surface.

Fig. 11. *T. intybacea.* From specimens collected in pine woods incrusting fallen pine leaves and twigs at Middlebury, Vt. *a* shows upper surface with matted, adnate squamules and whitish, thick, entire margin; *b*, the hymenium of lower surface.

Fig. 12. *T. griseozonata.* From specimen of type collection, distributed in Ravenel, Fun. Amer., No. 444.

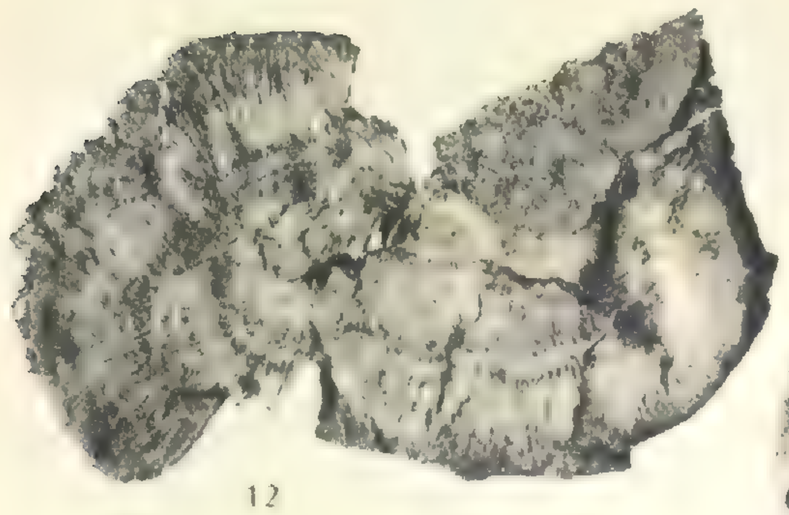
Fig. 13. *T. albido-brunnea.* *a*, upper side of specimen collected at Saugatuck, Mich., by E. T. and S. A. Harper, No. 654. The specimen is about 2 cm. thick; *b*, hymenium of specimen collected at Lake Dunmore, Vt.

Fig. 14. *T. cuticularis.* From specimens collected at Blue Mounds, Wis., by E. T. and S. A. Harper, No. 861. *a*, viewed obliquely from above; *b*, viewed from under side to show hymenium.

Fig. 15. *T. vialis.* From specimen collected at Lake Dunmore, Vt.

10

11



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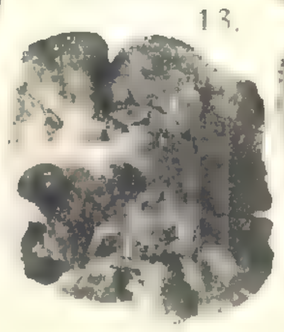

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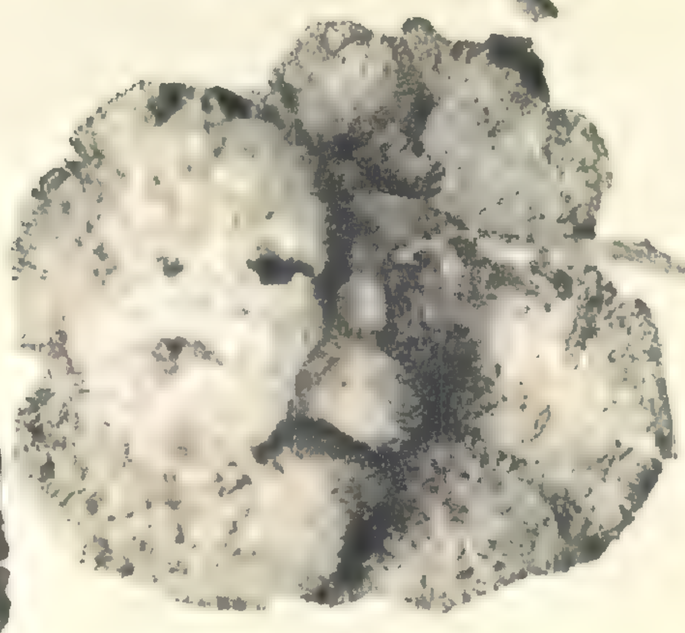
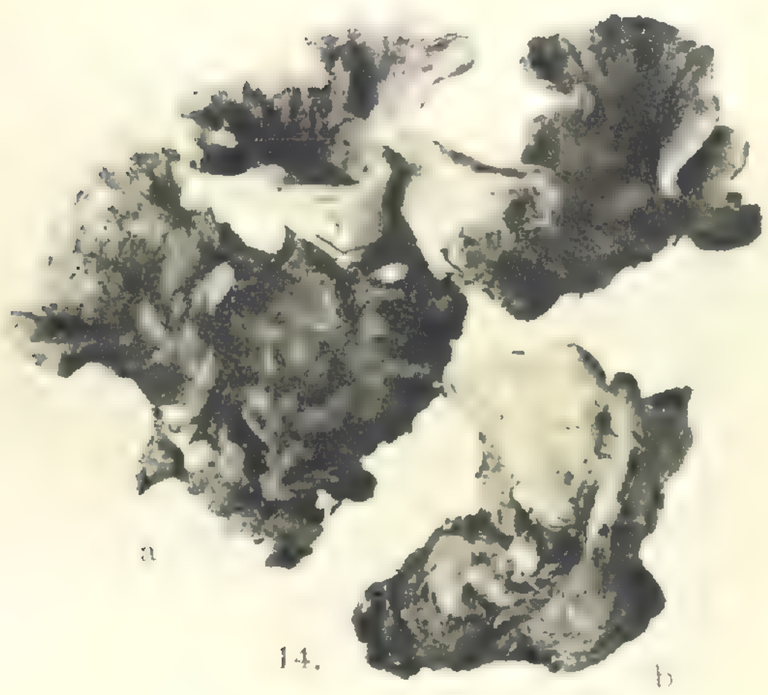


a

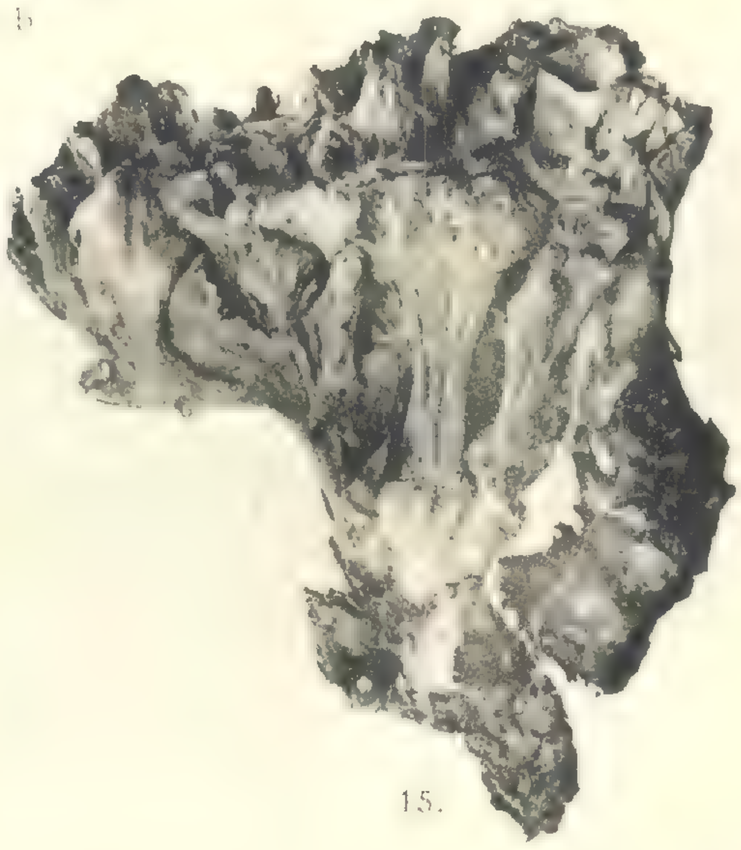
b



14.

a

b



15.

BURT — THELEPHORACEAE OF NORTH AMERICA

10. THELEPHORA TERRESTRIS.—11. T. INTYBACEA.—12. T. GRISEO-ZONATA.—13. T. ALBIDO-BRUNNEA.—14. T. CUTICULARIS.—15. T. VIALIS.

COCKAYNE, BOSTON.

INDICATIONS REGARDING THE SOURCE OF COMBINED NITROGEN FOR ULVA LACTUCA

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Introduction

Very little attention has been given the question of the sources of nitrogen for marine algæ. Nevertheless, the question is an interesting one both physiologically and ecologically, because of the extremely small amount of nitrogen supposed to be present in sea-water, and because of the very noticeable change in the type of algal flora when the nitrogen content of the environment is increased, as by the presence of sewage. The literature bearing on the subject is practically limited to a debate between a few authors as to the amount and form of nitrogen in sea–water, and the way in which the supply is maintained. This dispute involves some questions of fundamental importance for marine biology; consequently, a brief statement of the different views is pertinent.

Natterer (13) reports that careful analyses of water from the high seas show scarcely a trace of nitrates. Nitrites are somewhat more abundant, but not sufficiently so to admit of quantitative determination. Ammonium compounds, on the other hand, according to Thoulet, are present in sufficient amount to be quantitatively determined, and vary from .13 to .34 mg. per liter (.013–.034 per cent) according to the locality. Reinke (15) considers these amounts of nitrogen reported to be insufficient for the production of the enormous amount of living material in the sea, especially when the activity of nitrifying and denitrifying bacteria is taken into account. He considers as of prime importance in this question the nitrogen-fixing bacteria which have been demonstrated in sea-water by Benecke and Keutner (4), and others. Reinke found *Azotobacter* embedded in the gelatinous material on the surface of *Laminaria* fronds and argues for a symbiotic relation between the algæ and bacteria.

Brandt (7), however, attaches little or no importance to Reinke's view, and maintains that the nitrogen content of sea-water is determined by a balance between the activity of denitrifying bacteria, on the one hand, and the great amount of nitrogenous material carried to the sea by the rivers, on the other. Brandt (5, 6) considers that the nitrogen content of sea-water is at a "minimum" and is the limiting factor in the production of marine organisms. Considering especially the plankton life, he finds that the amount of plankton is proportional to the nitrogen content of the water, and correlates the comparative poverty of tropical seas in plankton life with the relatively greater activity of denitrifying bacteria in the warmer waters.

More recently Pütter (14) has reported that analyses of the water from the Gulf of Naples give per liter .18 mg. of nitrogen in nitrates and nitrites, and .56 mg. in ammoniacal nitrogen. Furthermore, he claims that these figures represent less than half the total combined nitrogen actually present in sea-water. In his opinion there is no need for considering the nitrogen content to be at a "minimum" since it is present in greater concentration than the carbon dioxide. It is impossible to say which of these views is the correct one, and further work in this field is much needed.

The above named authors incidentally assume that nitrogen is available for the algæ only in the form of nitrates or ammonium salts. This is entirely an a priori assumption, as no data are offered in support of such a view. On the contrary, it seems more likely that the algæ can use many organic nitrogen compounds. This would seem probable in the light of recent work which has been done on the fresh-water algæ.

In regard to the nutrition of the fresh-water forms we have departed far from the old idea that green plants are strictly autotrophic. Thus, by the work of Beyerinck (3), Charpentier (8), Chick (9), Artari (1, 2), and others, it has been established that many of the fresh-water algæ have, with respect to nitrogen, distinct saprophytic tendencies,—preferring organic to inorganic nitrogen. Artari, especially, has shown that several algæ (*Chlamydomonas*, *Stichococcus*, *Chlorella*, *Scenedesmus*, and others) can grow and retain the chlorophyll under completely

saprophytic conditions, as in solutions containing amino acids and glucose in the absence of light and carbon dioxide. In all these cases, however, growth is more rapid under so-called mixotrophic conditions, i. e., with both organic nitrogen and carbon present in addition to sunlight and carbon dioxide. Artari takes up the question of the relative value of different nitrogenous compounds, and shows that they vary greatly with different algæ,—some preferring peptones, others, amino acids and ammonium salts, and a few, nitrates. On the whole, the majority of forms investigated grow best in the presence of amino nitrogen. Many algæ, especially of this last class, are often found in water polluted with sewage or decaying organic matter.

Among the marine algæ, there is a more or less definite flora characteristic of sewage-polluted waters. Most conspicuous among the plants of this group are the species of *Ulva*. Letts and Richards (11), in their reports on sewage in British harbors, state that *Ulva latissima* grows in excessive quantities in polluted waters, and they find that the nitrogen content of this seaweed varies with the degree of pollution of the water. Cultural experiments conducted by Letts and Richards showed that *Ulva latissima* grows more rapidly in a mixture of sewage and sea-water than in pure sea-water alone.

Experimental

Preliminary experiments were made at the Woods Hole Laboratory to determine the sources of available nitrogen for *Ulva lactuca*. The algal material used in the experiments was collected at the mouth of an inlet where the water was at all times highly polluted with sewage. The cultures were maintained in the laboratory in glass tumblers containing 150 cc. of solution. When brought in, the fronds of *Ulva* were well rinsed in clean sea-water and cut into strips exactly 3 cm. in length and about 2 cm. wide. Three such strips were placed in each vessel, and the cultures kept at a temperature of 21°C. by placing the vessels in a tray of running water. In each case the solution was renewed at the end of 5 days. After 10 days the strips were again measured and the increase in length recorded.

Two main types of nutrient solution were used,—one (solution A) being natural sea-water, the other (solution B) being an artificial sea-water minus nitrogen. These stock sea-waters were made double strength and subsequently diluted by the addition of distilled water and the stock solution of the nitrogenous compounds to be tested. The following nitrogen compounds were used in the experiments: ammonium nitrate, urea, acetamid, sodium asparaginate, acetanilid, and dimethylanilin. Parallel experiments were run, adding these compounds to solution A and solution B.

Preliminary tests roughly determined the maximum nontoxic concentrations of these compounds when added to seawater to be:

Ammonium nitrate	0.011 gram molecular
Urea	0.010 gram molecular
Acetamid	0.250 gram molecular
Asparagin	0.080 gram molecular

The table presents the results of the experiments. All figures for concentrations represent fractions of gram molecules per liter, except in the case of dimethylanilin. Here the solubility was not known and the figures represent fractions of a saturated solution in distilled water at 20°C. In the column headed "growth" is recorded the increase in length in millimeters of the strips of *Ulva* after 10 days in the solution. In each case the figures for growth represent the average of three or more cultures. Checks show the growth in solutions A and B with no additional nitrogen.

It is apparent from the following table that, under the conditions of the experiment, ammonium nitrate and urea are considerably better nutrients for *Ulva* than the other compounds used. These two cause a marked increase in growth over that of the controls, in both the artificial and natural sea-waters. The nutritive value of these compounds was also indicated by the healthy appearance of the cultures. The algæ were of a deep green color, very turgid, and considerably curled by rapid growth. Judging from the growth and general appearance of the cultures, there is little choice between the nutrient values of ammonium nitrate and urea.

COMPARATIVE TABLE SHOWING GROWTH OF STRIPS OF ULVA IN VARIOUS NITROGEN-CONTAINING SOLUTIONS

Ammonium nitrate			Urea			Acetamid		
Conc.	Growth		Conc.	Growth		Conc.	Growth	
	Sol.* A	Sol.* B		Sol.* A	Sol.* B		Sol.* A	Sol.* B
Check	0.8	0.3	Check	0.8	0.3	Check	0.8	0.3
0.00005	1.0	0.5	0.0005	1.4	0.5	0.001	0.8	0.4
0.0001	1.4	1.5	0.001	1.6	0.6	0.005	1.0	0.4
0.0005	1.9	2.0	0.005	1.6	1.4	0.01	0.7	0.5
0.001	1.4	1.2	0.01	0.9	1.3	0.10	1.0	1.0

Sodium asparaginate			Acetanilid			Dimethylanilin		
Conc.	Growth		Conc.	Growth		Conc.†	Growth	
	Sol.* A	Sol.* B		Sol.* A	Sol.* B		Sol.* A	Sol.* B
Check	0.8	0.3	Check	0.8	0.3	Check	0.8	0.3
0.002	0.7	0.6	0.0005	0.6	0.5	0.002	0.3	0.4
0.01	0.9	0.2	0.0025	0.0	0.0	0.02	0.0	0.5
0.05	0.7	0.0	0.0125	0.0	0.0	0.10	0.0	0.0

* Sol. A = natural sea-water; sol. B = artificial sea-water.

† Conc. under dimethylanilin represents fractions of a saturated solution in distilled water at 20°C.

Acetamid has a somewhat lower nutrient value than ammonium nitrate or urea, but still it causes a greater growth than do the control solutions to which no foreign nitrogen was added. The alga in acetamid solutions appeared normal in every way. The results with the sodium asparaginate were rather unexpected. This compound is well known to be a good nutrient for many fungi and fresh-water algæ. For *Ulva*, on the other hand, sodium asparaginate appears to have no appreciable nutrient value. In no case did it cause any notable increase in growth, although the algal material appeared perfectly normal.

Acetanilid and dimethylanilin are in a separate class,—being decidedly toxic at all the concentrations used. At the lowest concentrations there was slight growth at first, but in ten days all cultures were dead and discolored. The results with these last two compounds are comparable to those obtained with similar substances by Czapek (10) and by Lutz (12) working on fungi and fresh-water algæ. They found that compounds having the nitrogen attached directly to a benzene nucleus are toxic.

Pure culture methods were not attempted on account of the brief time available for this work, and the question of the possible interaction of ammonifying bacteria is therefore pertinent. However, the rapid augmentation of growth upon the addition of the amido compounds, and the comparative absence of bacteria both suggest a direct absorption of these substances. Moreover, since rapid growth of the alga occurs in concentrations of the amido compounds considerably greater than the toxic limit for ammonium salts, and since, further, no evidence of toxicity of fairly strong solutions of urea and acetamid developed during the interval of these experiments, no support is given to the thought that ammonification may be an important factor. However, in further continuation of this work it is proposed to control this possibility by quantitative tests.

It seems probable from the facts brought out here, as well as from the work of Letts and Richards, that *Ulva* is not limited to an inorganic nitrogen supply, since growth occurs with urea or acetamid as the sole source of nitrogen, and, as Letts and Richards have shown, that it grows more rapidly in sewage-polluted water than in pure sea-water. Undoubtedly, further experiments would show that other organic compounds can supply available nitrogen for *Ulva*.

The results also indicate that for *Ulva*, at least, the amount of available nitrogen in the water is the limiting factor in growth. This is shown by the fact that growth is more rapid in sea-water containing additional nitrogen (ammonium nitrate, or urea) than in pure sea-water. The above mentioned results of Letts and Richards also point to the same conclusion, as does

the abundant growth of *Ulva* in nature in waters polluted with sewage.

In conclusion, the writer is pleased to express his thanks for the generous assistance given during this study by Prof. B. M. Duggar, under whose direction the work was carried out while occupying a research table maintained by Dartmouth College at the Marine Biological Laboratory, Woods Hole, Massachusetts.

Graduate Laboratory, Missouri Botanical Garden.

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THE EFFECT OF CERTAIN CONDITIONS UPON THE ACIDITY OF TOMATO FRUITS

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In a recent communication the senior author (4) has referred to the possibility that the total acid content of tomato fruits ripened at a temperature of 30°C., or above, may be related in some way to the failure of lycopersicin development at that temperature. It was determined that the "total acidity for green, ripening, and ripe fruits, grown under the same conditions, is unexpectedly uniform, amounting to .57 to .58 per cent citric acid." The fruits just referred to were of the same variety picked at the same time. The tests of acid content of incubated fruits were made later in the season, and these indicated a lower acidity than that of normally green or ripe fruits. At that time the requisite material was obtained from the Department of Horticulture, Cornell University.

During the past summer several varieties of tomatoes were grown in the Missouri Botanical Garden in order to furnish material for further pigment studies, and incidentally this material has enabled us to determine with greater care the acid content of tomato fruits, especially of different varieties, and likewise the comparative acidity of fruits direct from the field and of those of the same picking incubated for various intervals. The tests included below were made by pulping thoroughly a weighed quantity of the tissue (15 gm.), diluting with 150 cc. distilled water, employing for each titration 25 cc. of this solution diluted with distilled water to 50 cc., and titrating with n/10 NaOH, using phenolphthalein as indicator. Not less than two titrations were made in any case, and these were from one or more samples of tissue. The accompanying table

indicates the variety and condition of the fruit; quantities of n/10 NaOH required to neutralize; and the per cent of acidity in terms of citric acid.

TABLE SHOWING ACID CONTENT OF TOMATO FRUITS

Variety	Condition			Average no. of cc. of n/10 NaOH, to neutralize	Total per cent of acid as citric
	When picked	Interval or incubation	When titrated*		
Dwarf Stone	Ripe	0	Red	1.695	.52
Dwarf Stone	Half grown	0	Green	1.82	.56
Dwarf Stone	Half grown	Incub. 32° C. 10 days	Artif. yellow	2.135	.66
Dwarf Stone	Half grown	Lab. 24 days	Red	1.375	.42
Dwarf Stone	Half grown	Incub. 32° C. 10 days	Green	1.485	.46
Sparks' Earliana	Ripe	0	Red	1.695	.52
Sparks' Earliana	Half grown	0	Green	1.87	.58
Truckers' Favorite	Half grown	Incub. 32° C. 22 days	Artif. yellow	2.56	.79
Truckers' Favorite	Half grown	Lab. 24 days	Red	1.66	.51
Red Peach	Half grown	Incub. 32° C. 22 days	Artif. yellow	2.115	.65
Red Peach	Half grown	Lab. 24 days	Red	1.675	.52
Yellow Peach	Half grown	Incub. 32° C. 22 days	Artif. yellow	2.47	.76
Yellow Peach	Half grown	Lab. 24 days	Yellow	2.065	.64
Yellow Plum	Ripe	0	Yellow	2.12	.65
Yellow Plum	Half grown	0	Green	1.92	.59
Yellow Pear	Half grown	Incub. 32° C. 20 days	Artif. yellow	1.60	.49
Yellow Pear	Half grown	Lab. 24 days	Yellow	1.395	.43

* All fruits designated "red," "yellow," and "artificial yellow" were, at the same time, ripe.

The results above reported may not yet be as extensive as might be desired in order to follow closely the changes in acidity under different conditions; but they consistently point out certain relations of interest which may be briefly enumerated as follows: (1) A comparison of the acid content of green and normally ripened fruits was made, using Dwarf Stone, Sparks' Earliana, and Yellow Plum, all direct from the field. There were no marked differences between the green and ripe stages within the variety; yet the acidity of the green fruits of the red varieties in these tests is somewhat higher, while the acid content of the green fruits of the one yellow variety tested is somewhat lower. (2) Fruits of Dwarf Stone, Truckers' Favorite, Red Peach, Yellow Peach, and Yellow Pear which

were picked green and ripened in the incubator at 32–33°C. (10–22 days) exhibit a higher acid content than either those ripened on the vines or those ripened at the temperature of the laboratory. (3) There are considerable differences in the acidity of varieties, but judging from the results of these tests the normally ripened fruits of yellow varieties commonly contain as much acid as those of red varieties.

The several facts brought out by these tests render it obvious that there is now no sufficient evidence to justify relating pigmentation to total acidity. The acidity changes are, however, interesting in themselves, in these as well as in other fruits. No attempt was made to follow progressively any changes in acidity induced by conditions; but in titrating on one occasion, after an interval of two days, new samples of both red and yellow fruits which had been ripened in the laboratory, it was found that the acidity had noticeably declined since the previous titrations from the same lots of fruits.

We have reckoned the acidity of the tomato in terms of citric acid, as is customary. It should be noted, however, that while Bowman (3) and others report citric as the chief acid of the tomato, Albahary (1), on the contrary, gives .48 per cent as the malic acid content and .09 per cent as that of citric acid in the fresh fruits. The author last mentioned gives no indications respecting the variety or condition of the fruit employed. In a later contribution (2) he reports the results of analyzing tomato fruits in different stages of maturation, as follows: "1° le fruit vert avant l'apparition de la graine dans la pulpe; 2° le fruit vert au moment où la graine est complètement formée; 3° le fruit rouge arrivé à sa pleine maturation." In the second stage, corresponding to practically full grown, green, he finds .58, and in the ripe fruits .42 per cent of organic acids. This is in complete agreement with our findings. In the earliest stage of fruit development Albahary finds an acid content of only .116 per cent. Wehmer (5), after quoting Albahary (1) as to the percentage of the various acids in the fruit, remarks, "Die Acidität wechselt stark je nach dem Reifestadium (von 0,06–0,697% des Saftes auf Citronensäure berechnet)." He does not indicate the

source of these data, and certainly the smaller percentage given can refer only to the youngest stages of fruit development.

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A METHOD FOR THE DIFFERENTIAL STAINING OF FUNGOUS AND HOST CELLS

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In making histological studies of fungi on living or dead plant tissues the use of the stain known as "Pianeze IIIb" has been found very satisfactory in differentiating the fungus from the plant substratum, this differentiation occurring both in lignified and unlignified cell walls. The host tissue stains green and the mycelium a deep pink. This stain, devised by Dr. Pianeze for the study of cancer tissue,[1] is made up as follows:

Malachite green	0.50 gm.
Acid fuchsin	0.10 gm.
"Martius gelb"	0.01 gm.
Water, distilled	150.00 cc.
Alcohol, 95 per cent	50.00 cc.

Dr. Pianeze reports that it gives the following staining reactions: green in chromatin of resting or dividing nucleus, rose in cell protoplasm and membrane, and red in cancer bodies. For use with plant tissues the procedure is as follows: Wash in water or alcohol, stain in the undiluted mixture 15–45 minutes, remove excess stain in water, and decolorize in 95 per cent alcohol to which a few drops of hydrochloric acid have been added. For permanent mounts, clear with a carbol-turpentine mixture, remove clearer in xylol, and mount in balsam. Preparations of *Stereum*, *Corticium*, and *Polystictus* have been made with great success.

This stain is also valuable for staining germinated spores on the surface of a leaf. The procedure in this case is as follows: Infect marked portions of a leaf with a suspension of spores applied with a pipette, and place the plant under suitable conditions for fungous growth for 24–48 hours. Then permit

[1] Pianeze, G. Beitrag zur Histologie und Aetiologie des Carcinoms. Beiträge z. path. Anat. u. z. allg. Path. Supplement 1: 1–193. 1896. [cf. p. 58.]

the leaf to dry in the air, remove the area desired from the balance of the leaf, and place in a killing fluid. The best combined killing and tissue-clearing mixture for this purpose is one recommended by Dr. Duggar, composed of glacial acetic acid and 95 per cent alcohol. I have used equal parts of these agents most advantageously. This dissolves the chlorophyll, renders the leaf transparent or nearly so, and at the same time fixes the fungus with little plasmolysis. Allow the killing mixture to act for 24–36 hours; wash in 50 or 70 per cent alcohol, to remove the acid; and pass successively through the stain (15–30 minutes), water (2 minutes), acid alcohol (as short a time as possible), carbol-turpentine (until clear), xylol (until clearing agent is removed), and then mount in balsam. This process of differential staining has been successfully used with *Ascochyta Pisi* on pea, *Helminthosporium sativum* on barley, and *Phoma Brassicæ* on cabbage.

Pianeze's stain has not given as good results with the rusts as Durand's combination of Delafield's haematoxylin and eosin. Durand's stain[1] was not uniformly successful, however, and it was found that one of the chief difficulties often experienced finds its explanation in the killing solution which the stain follows. Flemming's solution, which was first used, gave very poor results. A modification of Gilson's mercuric chloride solution was found most satisfactory. This solution, as recommended by Dr. Durand, is made up as follows:

Water, distilled	60 cc.
Alcohol, 95 per cent	42 cc.
Acetic acid, glacial	18 cc.
Nitric acid, concentrated	2 cc.
Mercuric chloride, sat. aq. sol.	11 cc.

Diseased tissue may be fixed from 6 to 24 hours, then washed in 65 per cent alcohol, run through the alcohols, infiltrated with cedar oil, and imbedded in paraffin. This method is undesirable for nuclear structures, but gives excellent preparations for gross histological work.

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TWO TRUNK DISEASES OF THE MESQUITE

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The diseases of the mesquite (*Prosopis glandulosa* Torr.) hitherto recorded are comparatively few in number; Heald and Wolf (5) enumerate seven from southern Texas as due to fungi. The pods are frequently affected by an anthracnose, *Glœosporium leguminum* (Cke.) Sacc.; the leaves are attacked by *Cercospora prosopidis* Heald and Wolf, a species of powdery mildew (*Erysiphe* ?), and by a rust, *Ravenelia arizonica* Ell. & Ev.; and a leaf blight due to some unknown cause is also mentioned. The large limbs and smaller branches show galls, evidently not due to insect attack, and the mistletoe (*Phoradendron flavescens* (Pursh) Nutt. is sometimes destructive. In addition to the above, the writer has frequently noted the weakening effect, particularly near the ends of branches, brought about by vigorous growths of the ball moss (*Tillandsia recurvata* L.). Birge (1) has given a good description of the effects of this plant on trees in Texas.

Of the insect injuries of the mesquite, that of the mesquite borer (*Cyllene antennatus* White) is of interest. The insect is described by Horn (6) as attacking mesquite wood in Arizona, but no description of its work is given. While I have not seen the insect at work in Texas, the holes found in the mesquite trees are so like those described for other species of *Cyllene*, notably *Cyllene robiniæ* Forster (10)—which attacks the locust—that the assumption seems warranted that the Texas insect is the one referred to by Horn. The tunnels extend straight through the bark into the heart-wood, and up and down in the latter, thus forming ideal channels for the entrance of fungous spores.

The only reference to trunk diseases which has been found is a brief statement by Havard (4), in an account of the mesquite, in which he mentions that "unfortunately it too often happens that the zones of the heart-wood are fissured, decayed or de-

tached from each other, so that it is difficult to get flawless boards."

In 1912 the writer found the older mesquite trees in the vicinity of San Antonio, Texas, seriously affected by a trunk disease, caused by one of the polyporous fungi. In one small field some twenty or more trees were found bearing the fruiting bodies of this fungus. Its distribution in the vicinity of San Antonio was general, and it is probable that it extends over a wider range, as evidenced by the finding of a sporophore by Underwood in the vicinity of Austin, in 1891.

Where the mesquite develops into a bush with several trunks, sometimes only one of the several trunks is affected, but in other cases several or all of them contract the disease. The age of the affected trees was difficult to estimate. The mesquite grows rather rapidly at first, but very slowly after eight or ten years. According to Sargent, trunks thirty years old may be seven to eight inches in diameter, while trees one foot in diameter are probably over one hundred years old. The trees found affected were from two to ten inches in diameter and all over twenty years of age, some of them probably very much older.

The decay is confined entirely to the heart-wood of the main trunks, extending from the ground up into the trunk for varying distances. The distribution is such that it is obvious that the fungus gains entrance through wounds in the trunk above the ground, chiefly through old branch stubs and borer holes, as is so frequently the case with trunk diseases of this kind. One instance was found which made it appear obvious that the holes made by the borer had served to give the fungus a start.

Sections of diseased trunks showed that the heart-wood was decayed to a greater or less degree (pl. 6 fig. 2). Mesquite wood has very sharply defined heart and sap-wood. The latter is light yellow or almost white and very narrow, being composed of but a few rings of wood, whereas the heart-wood is rich brown or reddish. The decay of the heart-wood begins near the center, and gradually spreads outward towards the bark; there is very little, if any, change in color (except that the decayed wood is a lighter shade of brown), and here and there irregular, thin lines of undecayed wood can be seen extending through the diseased

part. The decayed wood is very brittle, but still remains fibrous, that is, it does not crumble into powder like charcoal. It splits like sound wood, but is spongy and soft. The wood of the mesquite is very hard and heavy, a cubic foot weighing 47.69 pounds when absolutely dry. It consists of numerous, distinct medullary rays, and distinct but irregularly distributed bands of very thick-walled wood fibers, between which occurs a thinner-celled wood parenchyma. In the heart-wood the lumina of the cells of the latter tissue are usually completely filled with a yellow-brown substance, largely composed of tannin. McMurtree (8) found tannic acid in large quantities in mesquite wood, 6.21 per cent in the heart-wood, 0.5 per cent in the sap-wood, and 0.5 per cent in the bark. Besides tannin he found of materials other than tannin, insoluble in water but extracted by ether, 0.6 per cent in the heart-wood, 6.7 per cent in the sap-wood, and 1.84 per cent in the bark. A considerable number of large, open ducts are found in the early part of each wood ring. These also are filled with a yellow-brown substance similar to that found in the wood parenchyma.

The fine, colorless mycelium of the fungus spreads throughout the wood substance. Unlike *Polyporus rimosus* in locust wood (10), the fungus does not destroy the wood as a whole, but attacks only the heavily lignified groups of wood fibers. These are wholly destroyed, leaving holes or gaps between the vessels and wood parenchyma. The dissolution of the wood fibers evidently proceeds with great rapidity, starting with the secondary thickening of each cell. The cells disappear entirely, and in advanced stages of decay small masses of mycelium are the only evidence of their former presence. Although the wood parenchyma and the vessels are filled with hyphæ, they resist destruction almost completely,—a fact which may be connected with the very high tannin content of both of these tissues. The recent results of Wehmer (11), who found that for certain species of fungi tannin exerts a retarding influence on development, and the similar findings of Knudson (7), and of Cook and Taubenhaus (3), who state that "tannin has a tendency to retard or inhibit the growth of fungi," and that "the parasitic forms are more sensitive to the action of tannin than the saprophytic forms," lend support to this idea. Cook and Taubenhaus

also found that for the parasitic fungi tested, concentrations of from 0.1 per cent to 0.6 per cent were sufficient to retard growth. While the mere presence of considerable tannin may not entirely prevent the development of a fungus, it may retard its growth, and in the mesquite may explain the comparative immunity of the wood parenchyma to its attacks. The selective destruction of the wood fibers will serve to distinguish this form of decay from the other types of hardwood decay.

From the material found it was not possible to judge of the ultimate stages of the disease. In view of the fact, however, that sporophores four years old were observed, it seems that the resistance of a part of the wood structure is more or less permanent. No mesquite trees were found broken off as a result of the action of the fungus. It is conceivable, however, that very severe storms might break off trees weakened by the disease.

The fungus which causes the decay is *Polyporus texanus* (Murrill) Sacc. & Trott. The sporophores, which are annual and very distinct and easily recognized, develop around old knots. At the end of one year the sporophore dries and cracks (pl. 6 fig. 1, and pl. 7 figs. 1, 2), and many of them become badly eaten by insects. The latter may completely destroy the fruiting structure, thereby preventing the formation of new pilei from the original one. The sporophores occur either singly or in groups. In the latter case the oldest sporophore of the group is situated near the trunk, and gives rise during the second year to another pileus; from the latter a third one may grow out during the following year. This habit is well shown in pl. 6 fig. 1, and in pl. 7 fig. 1. The photograph reproduced in pl. 6 fig. 1 shows a group of three sporophores from below; the oldest one (in the back), dried and cracked; the second one formed immediately below the oldest one; and the youngest one developed at the side. This condition is also evident in pl. 7 figs. 1, 2. On the trees observed there was usually only one sporophore or a single group of sporophores, and while the internal decay extended in some cases for ten to twelve feet up and down in the trunk, in no case did the sporophores develop at more than one point.

Polyporus texanus (Murrill) Sacc. & Trott., was first described by Murrill (9) in 1904 from a specimen collected by Under-

wood on a mesquite (?) tree near Austin, Texas, in 1891. Murrill's description of this fungus is as follows:

"Pileus ungulate, attached by the vertex, 3 x 5 x 4 cm., surface fulvous to fuliginous, concentrically and radially rimose, especially in age, the separated areas imbricated; margin very obtuse, concolorous, context corky, concentrically banded, fulvous to umbrinous, very thin, only one-tenth the length of the tubes in thickness; tubes 3 cm. long, 2–3 to a mm., tawny chestnut, polygonal, edges thin, entire; spores ovoid, smooth, very dark brown, 1–2 guttulate, 8 x 10μ."

While this description was made from one specimen, the characterization is a good one and well defines the sporophores recently collected, and now in the herbarium of the Missouri Botanical Garden. One of the marked characters of the fruiting structure is the concentrically and radially rimose surface (pl. 7 figs. 1, 2) with imbricated areas, particularly in the older specimens. The tubes are very long, 2–3$\frac{1}{2}$ cm. (as stated by Murrill), and make up the larger part of the mass of the sporophore. The largest specimen found measured 9.5 cm. in width, 7 cm. in length, and 5 cm. in thickness. Using Ridgeway's color scale, the top is avellaneous gray, the tubes tawny, the substance antique brown (umbrinus of Saccardo's scale); near the margin the color is verona brown to warm sepia. Murrill's statement that the sporophore is attached by the vertex should be amplified, as many of the sporophores are practically dimidiate. With the additional material now available for study, the modified description of the fungus in question is as follows:

Polyporus texanus (Murrill) Sacc. & Trott. Syll. Fung. **21**: 272. 1912.

Inonotus texanus Murrill, Bull. Torr. Bot. Club **31**: 597. 1905.

Pileus ungulate, attached by the vertex or dimidiate, 4–9.5 cm. wide, 3–7 cm. long, and 4–5 cm. thick; surface avellaneous gray to fulvous, concentrically and radially rimose, especially in age, the separated areas imbricated; margin very obtuse, verona brown to warm sepia; context corky, concentrically banded, antique brown, very thin, only one-tenth the length of the tubes in thickness; tubes 2–3$\frac{1}{3}$ cm. long, 2–3 to a mm., tawny, polygonal, edges thin, entire; spores ovoid, smooth, very dark brown, 1–2 guttulate, 8 x 10 μ. Parasitic on living mesquite trees.

In the same locality in which *Polyporus texanus* occurred, one mesquite tree was found bearing a sporophore of *Fomes rimosus* Berk. This fungus causes the heart rot of *Robinia Pseudo-Acacia* (10), and it is of interest to note its occurrence on a new host. The specimen found is a typical sporophore of *Fomes rimosus*, measuring about two inches in length; unfortunately it was not recognized at the time of collection, and sections of the affected tree were therefore not made. In view of the destructive character of this fungus when found on *Robinia*, however, it is probable that it causes a similar heart rot of the mesquite. Further search will be made in the San Antonio region for additional evidences of its occurrence.

The wood of the mesquite is usually described as being very resistant to decay after it has been cut from the tree. For many years mesquite posts have been used in the southwest in preference to other kinds. Mesquite ties, foundation posts, etc., have also proved that the wood is very resistant to decay. This applies only to the heart-wood, however. The sap-wood is very short-lived, and where small trunks are cut, as is now frequently the case, and used for fence posts, the length of life is very short, —sometimes not over two to three years. The destruction of the sap-wood is due to a number of insects and saprophytic fungi, all of which are common on dead branches, posts, etc., in the vicinity of San Antonio. Of the more common fungi, the following were recently collected: *Polystictus Lindheimeri* B. & C., *Stereum Leveillianum* Fr., *Schizophyllum commune* Fr., *Lenzites protractus* Fr., and *Stereum albobadium* Schw.

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Explanation of Plate

PLATE 6

Disease of the mesquite due to *Polyporus texanus*

Fig. 1. View showing the manner in which a group of sporophores of *Polyporus texanus* grows on the trunk; also the lower surfaces of the sporophores.

Fig. 2. Two sections of diseased mesquite trunk showing the manner in which the wood is destroyed.

FIG. 1.

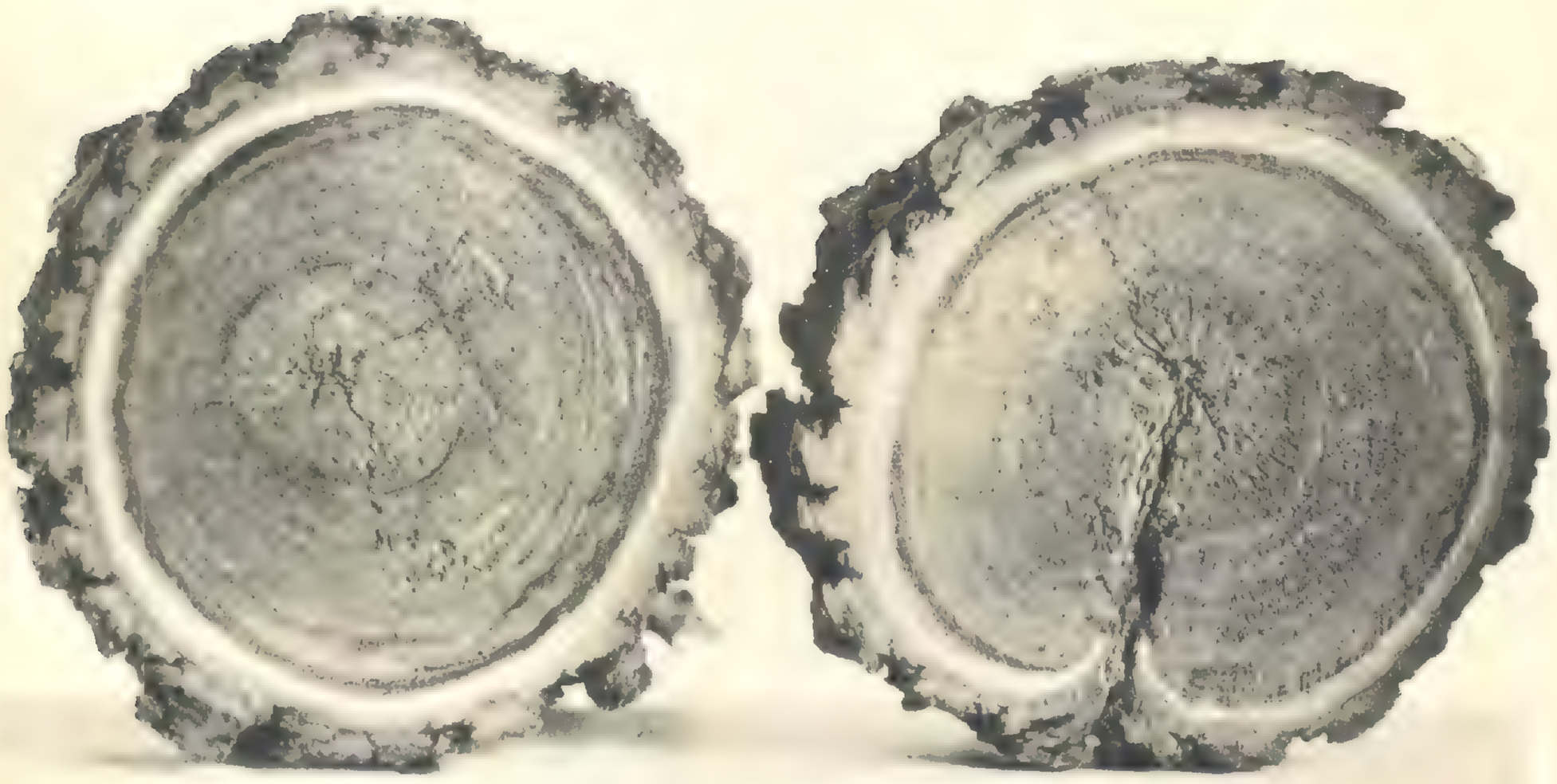

FIG. 2.

VON SCHRENK — TRUNK DISEASES OF MESQUITE

COCKAYNE, BOSTON.

Explanation of Plate

PLATE 7

Disease of the mesquite due to *Polyporus texanus*

Fig. 1. Side view of a group of sporophores of *Polyporus texanus* growing on a living mesquite tree.

Fig. 2. Front view of a group of sporophores of *Polyporus texanus* growing on a living mesquite tree.

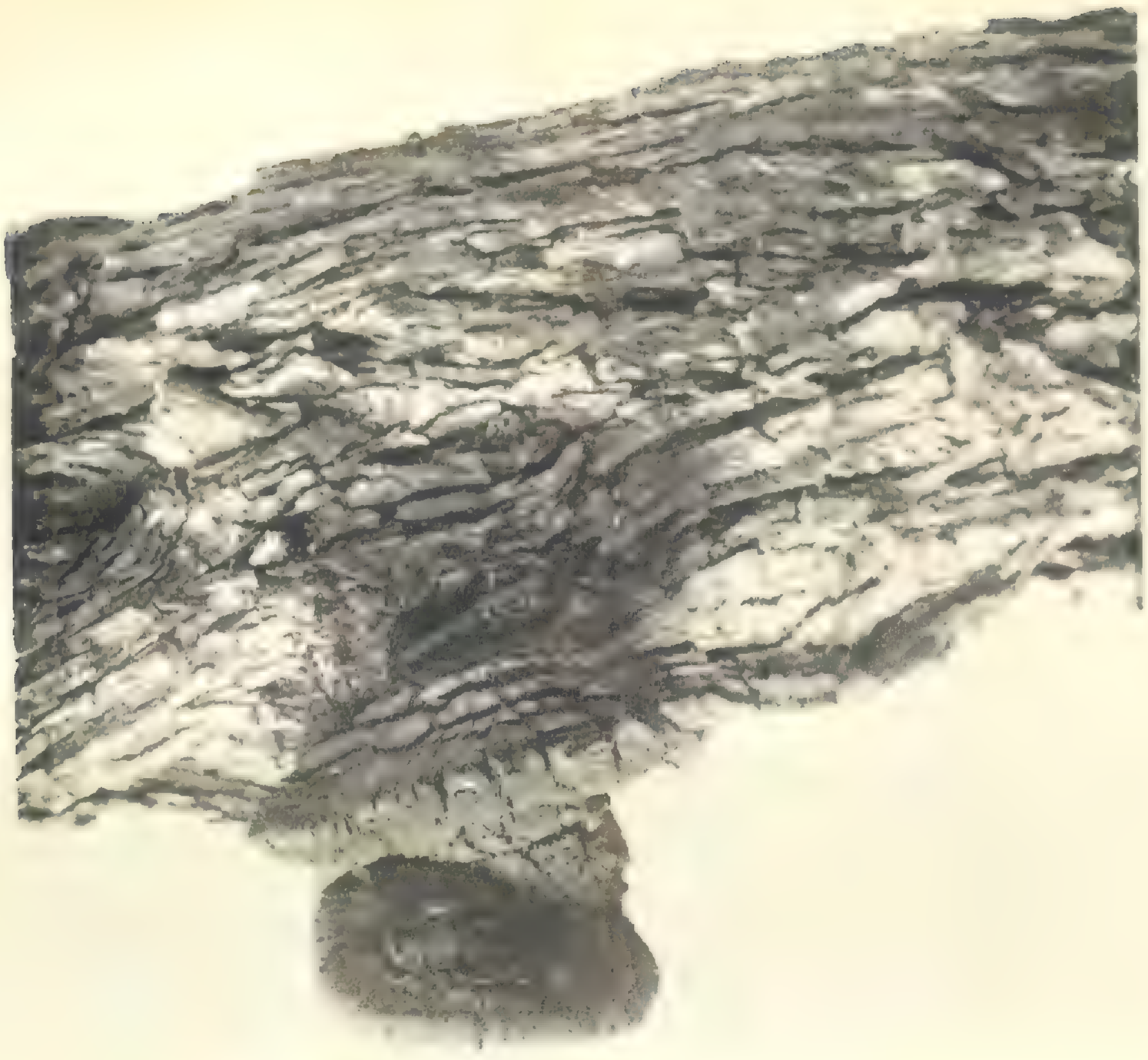

FIG. 1

FIG. 2

VON SCHRENK — TRUNK DISEASES OF MESQUITE

COCKAYNE, BOSTON.

A TRUNK DISEASE OF THE LILAC

HERMANN VON SCHRENK

Pathologist to the Missouri Botanical Garden

A general discussion of diseases of the common lilac (*Syringa vulgaris* L.) was recently published by Klebahn (3). This author enumerates a number of diseases, such as the one of bacterial origin ascribed to *Pseudomonas Syringæ*, various leaf diseases due to species of *Microsphæra*, *Glœosporium*, and other leaf parasites, and a disease due to *Botrytis cinerea*. The major part of the work, however, deals with a disease due to *Heterosporium Syringæ* Oud., affecting the leaves, and a serious twig blight due to *Phytophthora Syringæ* Klebahn. Subsequent papers by various writers deal with one or the other of the diseases mentioned by Klebahn.

During recent years a destructive trunk disease of the common lilac (*Syringa vulgaris* L.) has been noted a number of times in the Missouri Botanical Garden, and in grounds in the vicinity of St. Louis. The affected plants were usually old bushes which had been more or less neglected, and the tops of the leading trunks were frequently dead. Long shoots from the root and others from the part of the trunks near the ground made a dense tangle around the main stem; on the latter sporophores of *Polyporus versicolor* were found in various stages of development, sometimes isolated, but more frequently in groups. Sections were made of the trunks on which this fungus was growing and it was found that such trunks were invariably diseased, while those close by, either from the same root system or from adjacent bushes—which were free from the fungus—were always sound.

In pl. 8 fig. 1 two affected trunks are shown cut at points about three feet from the ground. In both cases the larger part of the stem was alive, as evidenced by the presence of vigorous shoots along the entire length. Pl. 8 fig. 2, and pl. 9 figs. 1, 2 represent sections of lilac trunks taken from different bushes to show different stages of the disease.

The wood of the lilac is white in color, hard, and close-grained. In younger trunks there is no appreciable difference between heart-wood and sap-wood; as the trunks grow older, however, the heart-wood turns darker, and in those twelve years old, or thereabouts, it is distinctly darker than the rather thin, white sap-wood.

The disease first manifests itself in the inner heart-wood, frequently in close proximity to the holes made by the lilac borer. This lepidopterous insect (*Podosesia syringæ* Harris) (for whose identification I am indebted to Dr. E. P. Felt) has been found very destructive to lilac bushes, and, according to Beutenmüller (1), occurs from New England and the middle states westward to Colorado and southwest to Texas. Quoting from Beutenmüller's account: "The female deposits her eggs in patches on roughened or knotty places on the bark of ash and lilac. The eggs, according to Hulst, hatch in about six days, and the newly born larvæ at once eat their way through the bark into the solid wood. They run their channels longitudinally for about 8–10 inches through the wood. The larvæ pupate in slight cocoons after cutting their way to the bark, of which they leave only a thin outer skin. The pupation usually takes place early in May, and the moths emerge in about three weeks." Felt (2) briefly described the habits of the larva, stating that "a sign of its presence in midsummer being largely the sudden wilting of a shoot." He quotes from an observation made by Dr. Kellicott in which the latter states that he "watched 20 or more issue from a single tree in one day, and found that often there were more than one hundred in one tree." Felt recommends cutting and burning all infested wood in the early spring.

In the vicinity of St. Louis the lilac borer has been very active in recent years, judging from the fact that very few lilac bushes over five years old were found free from its attacks. Without much doubt the fungous spores get into the interior of the lilac trunks through the borer holes, and start to develop within the heart-wood on the edges of the borer holes. In pl. 9 fig. 1 two borer holes, still filled with pieces of the borings, can be seen in the lower right-hand trunk, and one small hole in this same section occurs in the sap-wood. The fungus, after it has begun to

grow in the hole, rapidly spreads up and down in the heart-wood, and soon grows out from the center toward the bark. As the disease progresses, the wood is converted into a soft, pithy, white mass, having the consistency of corn-stalk pith. The line of demarcation between the sound and completely destroyed wood is very sharp (see pl. 9), resembling in this respect the type of decay caused by this same fungus in living catalpa trees ((5), pl. 26). The line between sound and decayed wood is so sharp that entirely decayed fibers adjoin perfectly sound ones. Between the wholly unaffected wood and the completely destroyed fibers, is a narrow ring of darker wood, which is, to all intents and purposes, sound; the wood cells are partially invaded by the mycelium of the fungus, and the lumina are filled with a yellow-brown liquid, which when seen in mass gives the section the dark color referred to. This liquid dries out in some places and leaves a brown amorphous substance, such as has frequently been found in the early stages of decomposition of hardwood wood fibers (6). It probably consists of decomposition products which are infiltrated into the sound wood immediately in advance of the fungus. In cases where the fungus starts in several centers, rings of the darker colored wood surround each decayed portion, a condition which is well shown in pl. 9 figs. 1, 2, where the fungus is growing in the center of the trunk and in addition in three more peripheral localities. In the lilac the brown substance referred to is ultimately destroyed (see the middle trunk of the lower tier, pl. 9 fig. 2, where the wood is destroyed up to the bark).

The completely decayed wood, which readily absorbs water, resembles pith, and in general is very similar to catalpa wood destroyed by *Polyporus versicolor* (5). It has some of the attributes of wood, i.e., it can be split, is fairly compact, and cannot be crumbled into powder. Sound lilac wood is very heavy and hard, and is composed almost wholly of very thick-walled wood cells, with small vessels scattered with considerable regularity throughout the annual ring; wood parenchyma is almost wholly absent. The hyphæ of *Polyporus versicolor* attack and very rapidly destroy the layers of secondary thickening of the wood cells. The middle lamellæ retain the nature of lignified fibers and resist destruction almost entirely, although

here and there some of them are dissolved, giving rise to small separated cell groups. Entire dissolution rarely takes place (this was also found to be true for diseased catalpa wood ((5) pl. 52)). With the removal of the secondary thickening, the resulting decayed wood has a skeletonized appearance. It has all of the elements, but these are very thin-walled. The fine medullary-ray cells are destroyed here and there, producing radial, isolated masses, but more frequently the decayed mass hangs together firmly. The dissolution of the layers of secondary thickening goes forward very evenly, bringing about the sharp dividing line between sound and decayed wood already referred to.

The only difference between the catalpa and lilac diseases is that in the catalpa the entire wood mass is skeletonized, whereas in the lilac hard areas of undestroyed wood fibers are left here and there, surrounded by decayed wood (pl. 9 figs. 1, 2). These masses are either entire rings (pl. 9 fig. 1) or irregular areas lying detached within the decayed parts, and represent portions of the heart-wood which for some reason have temporarily escaped total destruction; the wood fibers are filled with the yellow-brown substance, but do not otherwise differ from normal wood fibers. As the disease progresses, however, they are finally destroyed. This was made evident by the fact that in the upper parts of diseased trunks these immune areas were always found coexistent with the early stages of the disease, while lower down in the trunks, where the advanced stages of decay had been reached, they were practically absent. The temporary immunity may be due to the presence of more resistant groups of wood fibers, possibly also to a high concentration of decomposition products.

The development of the fungous mycelium from the center of the trunk out toward the bark differs radically from that of any other disease known to the writer. In most trees the destruction of wood by a fungus growing in the dead heart-wood is confined to the latter,—further growth ceasing as soon as the mycelium reaches the sap ring. As has been suggested by Münch (4), this is probably due to the fact that most mycelia of wood-destroying fungi require a balance between the amounts of oxygen and water contained in the wood fiber. Any undue

percentages of either may make the conditions unfavorable for further development.

In the lilac disease the fungus may grow outward concentrically in a regular manner (pl. 9 fig. 1). Very frequently, however, the fungus grows out into the sap ring at one side, at first slowly, then more rapidly. This is well shown in pl. 9 fig. 2, where four successive stages are represented by photographs. In the upper left-hand trunk the fungus has almost reached the bark, and in the three lower ones it has reached the bark and is gradually killing it. The probable explanation for this behavior is to be sought in the water content of the wood fibers. It was found that in many cases where the fungus actually grew up to the bark and through it, that on that side the lilac borer had been active; the wood fibers in the vicinity of the holes dried sufficiently to make growth possible for the mycelium, and as the destruction took place more drying occurred in adjacent areas until ultimately the whole sap region on that side was invaded and destroyed.

A number of water determinations were made of the wood fiber in the immediate vicinity of the growing mycelium, and the results compared with those obtained from normal sap-wood. In all cases the sap-wood about to be invaded was found to have a very much lower water content than the normal sap-wood. Unfortunately, it was impossible to get exact data which would indicate accurately the highest moisture content at which growth was possible; infected wood had obviously already reached and gone beyond that point, and as to sound new wood, even that which was near the borer holes, nothing could be postulated with certainty concerning its susceptibility or non-susceptibility to fungous attack. It would be an interesting problem to test the water susceptibility of *Polyporus versicolor* in its relation to lilac wood. It seems probable, however, that the drying out of one side of the trunks was at least one of the determining factors in the rather striking and exceptional method of growth of the fungus. Whether the fungus would eventually have destroyed the entire trunk it is impossible to state, because no such wholly destroyed trunks were found. There seems to be no reason, however, why this should not

occur; in fact, the right-hand trunk of the lower tier in pl. 9 fig. 2 has very little live wood left.

After the mycelium has reached the bark it grows through it, and fruiting bodies develop on the outside. The latter sometimes occur singly, but more commonly in linear groups parallel to the long axis of the trunk. Frequently one or more fruiting bodies grow out from the holes made by the borer. In the right-hand trunk in pl. 8 fig. 1 sporophores are shown growing out at the base of vigorous, live shoots; in the left-hand trunk the shoot is dead, having been killed during the year as the fungus invaded the wood from which the shoot was growing.

The sporophores found were typical of *Polyporus versicolor* L. This fungus is so common on the dead wood of various hardwoods that a detailed description is hardly necessary. It is interesting to note here that this is the second instance where this fungus attacks living plants. In the case of the catalpa the fungus grows vigorously only in the live tree; infected wood rarely, if ever, is decayed after it is cut from the tree. Many thousand posts of catalpa, the heart of which had been partially destroyed by *Polyporus versicolor*, have served as fence posts during the last ten years without showing a sign of decay of that part of the wood which was sound at the time of cutting. With the lilac it is different; the dead wood is just as subject to attack as is dead oak, beech, or gum wood.

The age at which lilac bushes are attacked has not been definitely determined. Those examined were about 15–20 years old ($2\frac{1}{2}$ inches in diameter). The trunk shown in pl. 8 fig. 2 was over thirty years old. It is probable that the disease is not serious until the bushes are ten or more years old, although this will depend somewhat on the rate of growth. Trunks $1\frac{1}{2}$–2 inches in diameter were frequently found diseased. The effect of the disease is to gradually kill the top of the trunk; side shoots then develop farther down, which in turn are killed by the fungus, and eventually the trunk is broken off by the wind or snow.

The prevention of the disease is possible by continued attention to the borers. A careful examination for the latter should be made in June or July, and if any are present these should be killed by means of a wire, and the holes—after antiseptic treatment with some coal-tar compound—plugged. Painting

or brushing the lower parts of trunks with whale oil soap, or its equivalent, should also prove of value. Wherever a diseased trunk is found it should at once be cut out and burned. All dead wood in the neighborhood of lilac bushes should be cleaned up, so that the chances for infection may be reduced.

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Explanation of Plate

PLATE 8

Trunk disease of lilac due to *Polyporus versicolor*

Fig. 1. View of two diseased lilac trunks showing the sporophores of *Polyporus versicolor*, and the manner in which living branches grow from diseased trunks.

Fig. 2. Sections of an old diseased lilac trunk showing the decayed heart-wood.

VON SCHRENK—TRUNK DISEASE OF LILAC

COCKAYNE, BOSTON.

Explanation of Plate

PLATE 9

Trunk disease of the lilac due to *Polyporus versicolor*

Fig. 1. Sections of three diseased trunks showing early stages of the disease.

Fig. 2. Sections showing progressive stages of the lilac trunk disease.

FIG. 1.

FIG. 2.

VON SCHRENK — TRUNK DISEASE OF LILAC

COCKAYNE — BOSTON.

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DESCRIPTIONS OF NORTH AMERICAN SENECIONEÆ[1]

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The following descriptions and notes are the results obtained from a critical study of material in several herbaria during the preparation of a monograph of the North American species of the genus *Senecio*. Some of the species here described have been in manuscript a number of years and a few of them have been withheld from publication, because of incomplete specimens, hoping that additional material might be brought together before publication. In many cases supplementary and substantiating material has been obtained from which it is now possible to make fairly complete diagnoses. In one or two instances a reconsideration of certain natural groups within the genus, in the light of recent collections, has made it possible to combine forms which formerly were taken to represent distinct species. Very few new species have resulted from recent collections, but there are still many regions, particularly in Central America, which are inadequately explored. The writer would welcome material in this genus from any part of North America in order that the geographical range of species may be recorded as accurately as possible in his forthcoming monograph. The sections indicated in parentheses immediately following the generic name are in accordance with my preliminary paper to which reference is made under the species.

[1] Issued September 30, 1914.

Senecio (§ **Aurei**) **hyperborealis** Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. **32**: 20. 1902, nomen.

S. resedifolius Hook. Fl. Bor. Am. **1**: 333. *pl. 117*. 1833, not Less.

Herbaceus perennis; caule simplice vel ramoso suberecto 1–2 dm. alto plus minusve foliaceo juventate glabro vel parce flocculoso-tomentuloso sæpe ad basin et in axillis foliorum persistenter lanato-tomentoso; foliis inferioribus petiolatis indivisis vel plerumque irregulariter lyrato-pinnatifidis 4–10 cm. longis 1–2.5 cm. latis, lobis remotis; foliis superioribus multum reductis sessilibus et bracteiformibus; capitulis paucis terminalibus radiatis 10–12 mm. altis 2–3.5 cm. (radii inclusis) diametro; floribus femineis 10–12, ligulis flavis 10–12 mm. longis ca. 2 mm. latis; disci flosculis numerosis; achæniis sæpe paulo hispidulis.

Specimen examined:

Canada: Arctic America, *Hooker* (Gray Herb.), TYPE.

Var. **columbiensis** (Gray) Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. **32**: 20. 1902, nomen.

S. resedifolius var. *columbiensis* Gray, Syn. Fl. **1**^{2}: 390. 1884.

Habitu formae typicæ; capitulis heterogamis, ligulis floris femineis quam squamis involucri paulo brevioribus; achæniis glabris.

Specimen examined:

British Columbia: Mucklung River, 25 July, 1882, *Mr. Mackay* (Gray Herb.).

Senecio (§ **Lobati**) **prolixus,** comb. nov.

S. diffusus Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. **32**: 20. 1902, nomen, not Linn. f.

Herbaceus perennis glabrus vel in axillis foliorum albotomentosus; caule tereti striato simplici vel ramoso erecto 2–5 dm. alto; foliis petiolatis vel sessilibus inferioribus lyrato-pinnatifidis petiolo incluso usque ad 15 cm. longis 1.5–5 cm. latis utrinque glabris, segmentis lateralibus oblongo-cuneatis cum sinis altis rotundatis disjunctis granditer dentatis, superioribus remotis sessilibus pinnatifidis sursum multum reductis; inflorescentiis laxe corymboso-cymosis 1–2.5 dm. diametro; capitulis circiter 1 cm. altis radiatis; involucris campanulatis parce calyculatis glabris; involucri squamis plerumque 21 lanceolatis vel lineari-lanceolatis 5–6 mm. longis acuminatis acutis; flosculis liguliferis ca. 13, ligulis oblongis 5–6 mm. longis flavis;

floribus disci numerosis 50–60; achæniis maturitate 2–3 mm. longis striatis glabris.

Specimens examined:

California (?): "Mohave Region," April-May, coll. of 1884, *J. G. Lemmon, 3130* (Gray Herb.), TYPE.

Arizona: Wickenburg, valley of the Hassayampa River, April, 1876, *Dr. Edward Palmer, 614* (Gray Herb. and Mo. Bot. Gard. Herb.).

The specimens cited may be looked for in herbaria under *S. multilobatus* Torr. & Gray, to which the species here proposed is related, but from which it differs in well developed specimens in the outline and size of the leaves, loose inflorescence, and larger heads with 21 instead of 13 involucral bracts. *S. prolixus* has rather more the aspect of *S. Breweri* Davy.

Senecio (§ **Tomentosi**) **appendiculatus** Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. **32**: 20. 1902, nomen.

S. neo-mexicanus Gray, Proc. Am. Acad. **19**: 55. 1883, in part; Syn. Fl. **1**^{2}: 392. 1884, in part, as to plant of Thurber.

Herbaceus perennis ubique plus minusve albo-tomentosus; caulibus subcæspitosis erectis 1.5–3 dm. altis striatis sæpe foliaceis; foliis radicalibus oblanceolatis vel oblongo-obovatis petiolo incluso 3.5–10 cm. longis 0.5–2 cm. latis dentatis ad basin in petiolum paulatim angustatis integris, eis caulinis petiolatis vel sessilibus 2–7 cm. longis ad basin plerumque ampliatis irregulariter dentatis subamplexicaulibusque; inflorescentiis terminalibus corymboso-cymosis 6–12-cephalis; capitulis 10–12 mm. altis radiatis; involucris campanulatis minute calyculatis; involucri squamis plerumque 21 lanceolatis 5–7 mm. longis acutis sparsissime tomentulosis; flosculis liguliferis ca. 13, ligulis flavis; floribus disci numerosis ca. 70; achæniis glabris.

Specimens examined:

New Mexico: Mule Spring, May, 1851, *Geo. Thurber, 280* (Gray Herb.), TYPE; Organ Mountains, Dona Ana Co., 25 April, 1907, *E. O. Wooton, 3370* (Mo. Bot. Gard. Herb.).

This species is related to *S. neo-mexicanus* Gray, to which it has been usually referred, but from which it differs in having a more leafy stem, undivided leaves, and with the stem-leaves commonly ampliated into a more or less dentate half-clasping base, and finally in having glabrous instead of hirtellous achenes.

Senecio (§ **Tomentosi**) **convallium** Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. **32**: 20. 1902, nomen.

Herbaceus perennis ubique sericeo-pubescentes; caulibus cæspitosis erectis 3 dm. altis; foliis inferioribus rosulatis petiolatis elliptico-lanceolatis vel oblongo-oblanceolatis 2.5–6 cm. longis 5–12 mm. latis acutis integris vel supra mediam partem pauci-dentatis basi longe cuneatis integriusculis juventate utrinque sericeo-pubescentibus ætate supra plus minusve glabratis, foliis superioribus spathulato-oblanceolatis angusti-petiolatis; inflorescentiis corymboso-cymosis paucicapitatis; capitulis circiter 1 cm. altis subradiatis; involucri bracteis 13–15 lineari-attenuatis 7–9 mm. longis acutis sparse sericeo-tomentulosis; floribus femineis subligulatis; floribus disci 30–35; achaeniis 3.5 mm. longis striatis glabris.

Specimen examined:

Utah: Rabbit Valley, altitude 2130 m., August, 1875, *L. F. Ward, 704* of the "U. S. Geological and Geographical Survey of the Territories" (Gray Herb.), TYPE.

The species here characterized has been hitherto confused with *S. canus* Hook., from which it is readily distinguished by the subsericeous pubescence and technical characters of the head.

Senecio (§ **Tomentosi**) **kernensis** Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. **32**: 20. 1902, nomen.

Herbaceus perennis ubique dense lanato-tomentosus; caule tereti erecto ca. 1 dm. alto; foliis inferioribus rosulatis petiolatis elliptico-oblongis vel oblongo-rotundatis 1–3 cm. longis 3–10 mm. latis apice obtusis vel rotundatis basi abrupte angustatis vel subtruncatis utrinque dense lanato-tomentosis, marginibus integris vel subcrenato-dentatis revolutisque, foliis superioribus bracteiformibus multum reductis; inflorescentiis terminalibus corymboso-cymosis paucicapitatis; capitulis 8–10 mm. altis radiatis 5–8 mm. (radii exclusis) diametro parce calyculatis; involucri squamis ca. 13 lineari-lanceolatis 5–6 mm. longis acutis floccoso-tomentulosis subglabratis; achaeniis glabris.

Specimen examined:

California: South Fork of Kern River, altitude 3760 m., September, 1875, *Dr. J. T. Rothrock, 334* of the "Explorations and Surveys west of the 100th Meridian" (Gray Herb.), TYPE.

Senecio (§ **Tomentosi**) **macropus** Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. **32**: 20. 1902, nomen.

S. arizonicus Gray, Syn. Fl. **1**[2]: 392. 1884, in part, as to plant of Rusby.

Radix robusta in sicco 2.5 cm. diametro; caulibus erectis usque ad 7.5 dm. altis glabris vel in axillis foliorum albo-tomentulosis striatis plus minusve purpurascentibus; foliis radicalibus petiolatis lyrato-pinnatifidis petiolo incluso 10–14 cm. longis 4–5 cm. latis, segmentis paucijugis inæqualibus terminali majore ovato-oblongis 5–6 cm. longis grosse dentatis, ceteris cuneatis et dentatis vel linearibus et integris; foliis caulinis remotis sessilibus pinnato-lobatis semiamplexicaulibusque sursum sensim reductis; inflorescentiis terminalibus corymboso-cymosis; capitulis ca. 1 cm. altis radiatis, ligulis flavis; involucris campanulatis minute calyculatis; involucri squamis circiter 21 lineari-lanceolatis 6.5–8 mm. longis acutis glabris maturitate retrorsis; floribus disci numerosis; achæniis glabris.

Specimen examined:

Arizona: without definite locality, coll. of 1883, *H. H. Rusby, 175* (Gray Herb.), TYPE.

Professor Rusby's plant was referred by Dr. Gray to *S. arizonicus* Greene, but from the very large root, the sublyrate, smooth and even somewhat glaucous radical leaves, and nearly naked stem it seems amply distinct.

Senecio (§ **Tomentosi**) **oreophilus** Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. **32**: 20. 1902, nomen.

S. neo-mexicanus Gray, Proc. Am. Acad. **19**: 55. 1883, in part; Syn. Fl. **1**[2]: 392. 1884, in part, as to plant of Greene.

Herbaceous perennis juventate ubique tomentulosus denique plus minusve glabratus; caule tereti erecto striato 2–3 dm. alto subnudo 2–3-bracteato; foliis rosulatis petiolatis oblongo-oblanceolatis vel oblongo-cuneatis petiolo incluso 3–10 cm. longis 0.7–2.5 cm. latis supra mediam partem crenato-dentatis basi in petiolum sensim angustatis integriusculis juventate utrinque albo-tomentulosis mox glabratis; bracteis caulinis linearibus apice basique parum ampliatis dentatisque; inflorescentiis laxe corymboso-cymosis usque ad 1 dm. diametro; capitulis 10–12 mm. altis calyculatis radiatis; involucris campanulatis basi tomentulosis ceteris glabris; involucri squamis plerumque 21

lanceolatis 6.5–8 mm. longis acutis; flosculis liguliferis ca. 12, ligulis oblongis 8 mm. longis 3 mm. latis 4–5-nerviis; floribus disci numerosis ca. 50; achæniis in angulis sursum hispidulis.

Specimen examined:

New Mexico: Pinos Altos Mountains, 6 May, 1880, *Edward Lee Greene* (Gray Herb.), TYPE.

A plant similar in habit to *S. neo-mexicanus* Gray, to which Dr. Greene's specimen was referred by Professor Gray in establishing that species. A careful study of all the original material, which has been made possible through the courtesy of Dr. B. L. Robinson, has shown that the *S. neo-mexicanus* of Dr. Gray consisted of at least three recognizably distinct forms of which Wright's No. 1415, as the first specimen cited, must be taken as the type. With the Wright plant several specimens at hand are almost the exact counterpart. The Greene plant in question, namely *S. oreophilus*, differs in several important particulars, notably in its essentially naked stem, oblong-cuneate leaves with subentire or sinuate-dentate margin, and a marked tendency for the foliage to become glabrous with age.

Senecio (§ **Tomentosi**) **oreopolus** Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. **32**: 20. 1902, nomen.

Plate 11.

Herbaceus perennis ubique albo-tomentosus; caulibus cæspitosis simplice vel ramosis 0.8–3 dm. altis; foliis inferioribus petiolatis ovato-ellipticis vel elliptico-lanceolatis vel rarius subobovatis 0.8–3.5 cm. longis 5–18 mm. latis obtusis vel supra mediam partem paucidentatis basi abrupte vel longe cuneatis integriusculis juventate utrinque albo-tomentosis ætate supra paululo subinde glabratis, petiolatis 1–6.5 cm. longis, foliis supremis grosse reductis petiolatis vel sessilibus integris vel rarius irregulariter dentatis basi sæpe expansis et subauriculatis; inflorescentiis corymboso-cymosis; capitulis plerumque ca. 1 cm. (8–14 mm.) altis radiatis parce calyculatis; involucri squamis plerumque 13 (9–13) lanceolatis vel lineari-lanceolatis 5–7 mm. longis acutis glabris vel leviter tomentulosis; flosculis liguliferis 5–13; floribus disci 20–30; pappi setis albis bracteis involucri longioribus; achæniis 3–3.5 mm. longis glabris.

Specimens examined:

California: Rock Creek Cañon, Basin of the Upper Kern River, Tulare Co., altitude 3050 m., July, 1904, *H. M. Hall & H. D. Babcock, 5526* (Gray Herb.), TYPE; Natural Bridge, Volcano Creek, Basin of the Upper Kern River, altitude 2285 m., July, 1904, *H. M. Hall & H. D. Babcock, 5433* (Gray Herb.); gravelly slopes, Little Kern River, altitude 3045–3350 m., April-September, 1897, *C. A. Purpus, 5240* (Gray Herb. and Mo. Bot. Gard. Herb.); Castle Peak, near the highest point, altitude 2740 m., 5 August, 1903, *A. A. Heller, 7102* (Gray Herb. and Mo. Bot. Gard. Herb.); Sierra Nevada, coll. of 1875, *John Muir, 4452* (Mo. Bot. Gard. Herb.); near the summit of Silver Mountain, altitude 3350 m., coll. of 1863, *W. H. Brewer, 2050* (Gray Herb.); Ebbett's Pass, *W. H. Brewer, 2005* (Gray Herb.); Sonora Pass, *W. H. Brewer, 2686* (Gray Herb.); Mono Pass, coll. of 1866, *H. N. Bolander, 6140* (Gray Herb.).

Nevada: Mt. Rose, Washoe Co., altitude 3200 m., 26 August, 1911, *A. A. Heller, 9882* (Mo. Bot. Gard. Herb.).

Forma **aphanactis,** forma nova.

Caulis circiter 1 dm. altus; foliis petiolo incluso 1.5–2.5 cm. longis 5–7 mm. latis; capitulis discoideis.

Specimen examined:

California: mountain peak near Sonora Pass, altitude 3200 m., coll. of 1863, *W. H. Brewer, 1905* (Gray Herb.), TYPE.

Senecio (§ **Tomentosi**) **Wrightii** Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. **32**: 20. 1902, nomen.

S. fastigiatus Gray, Pl. Wright. ii. 99. 1853, not Nutt.

Herbaceus perennis ubique subtomentosus; caule erecto 1–4 dm. alto foliato; foliis oblongo-oblanceolatis vel lanceolatis indivisis et integris vel supra mediam partem paucidentatis juventate albo-tomentosis plus minusve glabratis, inferioribus basi integriusculis in petiolum sensim angustatis, eis caulinis sessilibus basi sæpius ampliatim et irregulariter dentatis amplexicaulibusque; inflorescentiis terminalibus subcorymboso-cymosis multicapitatis; capitulis 8–10 mm. altis minute calyculatis radiatis; involucris campanulatis basi subincrassatis tomentosis, bracteis involucri plerumque 13 lanceolatis 5–7 mm. longis acutis tomentulosis; flosculis liguliferis 6–8, ligulis anguste

oblongis ca. 8 mm. longis 4–5-nerviis; floribus disci ca. 30; achaeniis glabris.

Specimens examined:

New Mexico: ravines between the copper mines and the Mimbres, October, 1851, *Charles Wright, 1289* (Gray Herb. and Mo. Bot. Gard. Herb.), TYPE; Santa Rita del Cobre, 22 September, 1880, *E. L. Greene* (Mo. Bot. Gard. Herb.); among spruce, Lookout Mine, Sierra Co., altitude 2680 m., *O. B. Metcalfe, 1179* (Mo. Bot. Gard. Herb.).

Senecio (§ **Amplectentes**) **subauriculatus** Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. **32**: 21. 1902, nomen.

Plate 14.

Herbaceus perennis; caule erecto ramoso striato glabro; foliis in partibus superioribus caulinis anguste lanceolatis 5–15 cm. longis 0.5–1.5 cm. latis acuminatis acutis integris vel remote apiculato-denticulatis sessilibus et auriculo-semiamplexicaulibus vel basi in petiolum sensim angustatis et subdecurrentibus membranceis supra glabris juventate subtus floccoso-tomentosis denique plus minusve glabratis; inflorescentiis terminalibus laxe subcorymboso-cymosis; pedunculis bracteatis, bracteis lineari-attenuatis; capitulis radiatis 12–14 mm. altis heterogamis; involucris campanulatis calyculatis albo-floccoso-tomentulosis, bracteolis calyculatis linearis acutis suberoso-marginatis; involucri squamis plerumque 21 lineari-lanceolatis ca. 1 cm. longis acutis et atro-penicillatis; flosculis liguliferis ca. 13, ligulis oblongis flavibus; floribus disci numerosis (50–60); pappi setis albis; achæniis pubescentibus.

Specimen examined:

Mexico: State of Oaxaca, mountains southeast of Miahuatlan, altitude 2750–3170 m., coll. of 1895, *E. W. Nelson, 2526* (Gray Herb.), TYPE.

A well marked species related to *S. Warszewiczii* A. Br. & Bouché and to *S. prionopterus* Rob. & Greenm.

Senecio (§ **Mulgedifolii**) **alatipes** Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. **32**: 21. 1902, nomen.

Herbaceus perennis ubique glabrus; caule tereti striato erecto 1 m. vel ultra alto; foliis parte inferiori ignotis, eis caulinis petiolatis vel sessilibus amplexicaulibusque oblongo-ovatis vel oblongo-lanceolatis 0.5–1.5 dm. longis 2–5 cm. latis acutis vel

acuminatis indivisis vel subpanduriformibus utrinque glabris subtus pallidoribus, margine irregulariter calloso-dentatis; petiolis usque ad 12 cm. longis anguste alatis; inflorescentiis terminalibus paniculatis; capitulis 8–10 mm. altis discoideis 20–25-floris; involucris anguste campanulatis calyculatis glabris; involucri squamis plerumque 13 lineari-lanceolatis acutis penicillatis ca. 6 mm. longis; achæniis striatis glabris.

Specimen examined:

Mexico: State of Chiapas, between Teneapa and Yajalon, altitude 900–1520 m., 13 October, 1895, *E. W. Nelson, 3277* (U. S. Nat. Herb., fragments and tracing in Gray Herb.), TYPE.

Senecio (§ Mulgedifolii) **callosus** Schz. Bip. in Flora **28**: 498. 1845.

S. eximius Hemsl. Biol. Cent.-Am. Bot. **2**: 239. 1881, as to synonomy.—*S. doratophyllus* Hemsl. *l. c.*, in part, as to Bourgeau's No. 1086, not Benth.—*S. viejensis* and *S. latipes* Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. **32**: 21. 1902, nomen.—*Cacalia Toluccana* DC. Prodr. **6**: 328. 1837.—*C. prenanthoides* Gray, Proc. Am. Acad. **19**: 53. 1883, in part, as to Bourgeau's No. 1086, not HBK.—*Erechthites runcinata* Hemsl. Biol. Cent.-Am. Bot. **2**: 234. 1881, in part, as to Bourgeau's No. 1086, not DC.

Herbaceus perennis ubique glabrus vel sparsissime tomentellus; caule tereti erecto circiter 1 m. alto striato plus minusve purpurascenti; foliis radicalibus et eis caulinis infimis petiolatis vel sessilibus amplexicaulibusque runcinato-pinnatifidis, lobis remotis, usque ad 4 dm. longis 3–18 cm. latis utrinque glabris subtus pallidioribus calloso-dentatis, summis sessilibus et auriculato-amplexicaulibus indivisis lanceolato-attenuatis; inflorescentiis terminalibus laxe paniculatis polycephalis; capitulis discoideis 10–12 mm. altis calyculatis 15–34-floris; involucri squamis plerumque 13 (8–13) lineari-lanceolatis 8–10 mm. longis acutis glabris et corollis plus minusve purpurascentibus; pappi setis albis; achæniis striatis glabris.

Specimens examined:

Mexico: State of Mexico, Désierto Viejo pres Mexico, *Bourgeau, 1086* (Gray Herb. and Berlin Herb.); near Guapimalpam, coll. of 1855, *Schaffner* (Gray Herb.); fir woods, Sierra de las Cruces, 11 December, 1892, *C. G. Pringle, 5313* (Gray

Herb.); Sierra de las Cruces, altitude 3350 m., 11 February, 1899, *C. G. Pringle, 7709* (Mo. Bot. Gard. Herb.); Mt. Ixtaccihuatl, altitude 2430–3350 m., *C. A. Purpus, 100* (Gray Herb.); fir forests, Mt. Ixtaccihuatl, altitude 3350–3650 m., February, 1903, *C. A. Purpus, 45* (Mo. Bot. Gard. Herb.). State of Vera Cruz, Las Vigas, near Jalapa, 2 December, 1903, *C. G. Pringle, 11869* (Gray Herb.), *forma*. State of Oaxaca, without definite locality, *Cuming* (Gray Herb.). State of Colima, coll. of Jan. 9–Feb. 6, 1891, *Dr. Edward Palmer, 1145* (Gray Herb.), distributed as "*Erechthites runcinata* DC."

The examination of a large suite of herbarium specimens, particularly in the light of recently acquired material, has led the writer to place a somewhat different interpretation on this species than formerly; hence, a brief description is here given and a few specimens from widely distributed exsiccati, well illustrating the species, are cited.

Senecio (§ **Mulgedifolii**) **Coulteri** Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. **32**: 21. 1902, nomen.

Cacalia runcinata Less. Linnæa **5**: 162. 1830, not HBK.

Herbaceus perennis; caulibus erectis 3–6 dm. altis striatis paulo tomentulosis plus minusve purpurascentibus; foliis inferioribus petiolatis runcinato-pinnatifidis usque ad 3 dm. longis 1.5–6 cm. latis supra glabris subtus arachnoideo-tomentulosis inæqualiter et obtuse calloso-dentatis, foliis superioribus gradatim reductis sessilibus amplexicaulibusque; inflorescentiis terminalibus subcorymboso-cymosis; capitulis numerosis discoideis ca. 1 cm. altis brevi-calyculatis; bracteis involucri plerumque 13 lanceolatis acutis 8 mm. longis glabris et purpurascentibus; floribus disci 30–40; achæniis glabris.

Specimens examined:

Mexico: State of Vera Cruz, Real del Monte, *Dr. Thomas Coulter, 429* (Gray Herb.), TYPE, *C. Ehrenberg, 381* (Berlin Herb. and Gray Herb.); Mt. Orizaba, *Schiede, 363* (Berlin Herb.). State of Mexico, on Nevada de Toluca, 15 October, 1903, *J. N. Rose & J. N. Painter, 7940* (U. S. Nat. Herb. and Gray Herb.).

Senecio (§ **Mulgedifolii**) **iodanthus** Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. **32**: 21. 1902, nomen. Plate 12.

Herbaceus perennis; caulibus 5–9 dm. altis foliaceis striatis glabris plus minusve purpurascentibus; foliis inferioribus plerumque lyrato-pinnatifidis oblongo-lanceolatis 1.5–3 dm. longis 3.5–9 cm. latis acutis vel acuminatis sinuato-calloso-dentatis supra glabris subtus juventate arachnoideo-tomentulosis et sæpe crispo-puberulentis fere glabratis, foliis superioribus sursum gradatim reductis sessilibus amplexicaulibusque; inflorescentiis racemoso-paniculatis 2–5 dm. longis 0.3–1.2 dm. latis; capitulis 10–12 mm. altis discoideis calyculatis; bracteis involucri circiter 13 lanceolatis 8 mm. longis acutis vel obtusis penicillatis glabris vel sparse puberulentis purpurascentibus; floribus disci ca. 24; pappi setis albis quam corolla brevioribus; corollis albis vel purpurascentibus; achæniis glabris.

Specimens examined:

Mexico: State of Mexico, in pine woods, Nevada de Toluca, altitude 3000–3600 m., 26 September, 1892, *C. G. Pringle, 4302* (Gray Herb. and Mo. Bot. Gard. Herb.), TYPE. State of Morelos, Tres Marias Mts., altitude 2895 m., 5 November, 1903, *C. G. Pringle, 11498* (Gray Herb.).

This species is closely related to *S. Coulteri* Greenm. but differs in having a smooth and more leafy stem, nearly glabrous leaves, and distinctly racemose-paniculate inflorescence.

Senecio purpurascens Klatt, Leopoldina, Heft 24, p. 126. 1888.

Var. **fossanervius** Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. **32**: 21. 1902, nomen.

Formæ typicæ habitu simili; foliis inferioribus petiolatis, petiolo incluso, usque ad 11 cm. longis 1.5 cm. latis sinuato-dentatis vel ad basin sublyratis supra glabris fossanerviis subtus tomentellis et in nerviis pilosis; involucri squamis fere glabris.

Specimen examined:

Mexico: without definite locality, *E. W. Nelson, 1308* in part (U. S. Nat. Herb., fragments in Gray Herb.), TYPE.

Senecio (§ **Suffruticosi**) **carnerensis** Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. **32**: 21. 1902, nomen.

Perennis basi suffrutescens ubique plus minusve lignescens; caule tereti erecto simplici vel ramoso; foliis indivisis petiolatis vel sessilibus lanceolatis vel oblanceolatis 1.5–5 cm. longis usque ad 1 cm. latis acutis denticulatis juventate utrinque tomentosis

supra plus minusve glabratis subtus persistenter albo-tomentosis, superioribus subauriculatis; inflorescentiis terminalibus paucicapitatis; capitulis ca. 1 cm. altis brevi-calyculatis radiatis; bracteis involucri plerumque 13 anguste lanceolatis apice atratis acutis glabris vel parce tomentulosis; flosculis liguliferis plerumque 8, ligulis flavis 4-nerviis; floribus disci 30–40 achæniis sursum brevi-sericeo pubescentibus.

Specimen examined:

Mexico: State of Coahuila, mountains, Carneros Pass, altitude 3050 m., 8 September, 1889, *C. G. Pringle, 2857* (Gray Herb., photograph in Mo. Bot. Gard. Herb.), TYPE.

This species was originally referred to *S. longilobus* Benth., but it is more closely allied to *S. stoechadiformis* DC. and *S. Picridis* Schauer; it is readily separated from both these species by having fewer involucral bracts, short, appressed and black-tipped bracteoles suggesting those of *S. vulgaris* L.

Senecio (§ **Suffruticosi**) **filicifolius** Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. **32:** 21. 1902; Contr. U. S. Nat. Herb. **16:** 19. 1912, nomen.

Herbaceus perennis (?) erectus ramosus 1.5–4 dm. altus ubique glabrus; caule tereti ad basin plus minusve lignescenti; ramis ramulisque striatis stramineis; foliis sessilibus vel subalato-petiolatis pectinato-pinnatifidis 1.5–8 cm. longis 1–6 cm. latis; segmentis linearis attenuatis acutis; inflorescentiis subcorymboso-cymosis oligocephalis; capitulis ca. 12 mm. altis ligulatis; involucris campanulatis calyculatis; involucri squamis plerumque 21 bracteolis calyculatis duplo longioribus lineari-lanceolatis acutis glabris vel juventate parce tomentulosis mox glabratis; flosculis liguliferis ca. 12, ligulis flavis; floribus disci 50–60; pappi setis albis; achæniis sursum sericeo-hispidulis.

Specimens examined:

Arizona: Valley of the Santa Cruz River, 11 May, 1881, *C. G. Pringle, 316* (Gray Herb.), TYPE; Tucson, 12 March, 1892, *J. W. Toumey, 708* (Gray Herb.); Tempe, coll. of 1892, *Ganong & Blaschka* (Gray Herb.); Hart's Ranch, 17 miles south of Tucson, 11 April, 1903, *J. J. Thornber, 436* (Mo. Bot. Gard. Herb.); Ft. Huachuca, coll. of 1894, *Maj. T. E. Wilcox* (Mo. Bot. Gard. Herb.); open cañons, San Francisco Mts., April, 1887, *H. H. Rusby, 214* in part (Mo. Bot. Gard. Herb.).

Mexico: Sandy plains near Altar, State of Sonora, 4 April, 1884, *C. G. Pringle* (Gray Herb.).

This species has been hitherto included with *S. Douglasii* DC. from which it differs in being essentially glabrous throughout, in having usually more numerous and shorter lateral leaf-segments, fewer, shorter, and less conspicuous calyculate bracteoles.

Senecio (§ **Suffruticosi**) **teliformis** Greenm. Monogr. Senecio, pt. 1, 26. 1901; in Engl. Bot. Jahrb. **32**: 22. 1902, nomen.

Herbaceus perennis; caule erecto tereti superne striato stramineo floccoso-tomentoso plus minusve glabrato; foliis supremis sessilibus lanceolato-attenuatis 3–6 cm. longis ad basin ampliatis usque ad 1.5 cm. latitudine semiamplexicaulibusque supra juventate floccoso-tomentulosis plus minusve glabratis subtus persistenter albo-tomentosis, margine dentatis vel denticulatis revolutisque; foliis inferioribus ignotis; inflorescentiis terminalibus corymboso-cymosis multicapitatis bracteatis floccoso-tomentulosis; capitulis 8–10 mm. altis radiatis calyculatis, bracteolis calyculatis lineari-attenuatis conspicuis subflaccidis floccoso-pubescentibus; involucri bracteis plerumque 21 linearilanceolatis 5–6 mm. longis acutis glabris penicillatis; flosculis liguliferis sæpius 8, ligulis oblongis 5–6 mm. longis flavis; floribus disci ca. 40 quam bracteis involucri longioribus, pappi setis albis; achæniis sursum adpresso-sericeo-pubescentibus maturitate 3 mm. longis.

Specimen examined:

Mexico: State of Oaxaca, mountains of Telixtlahuaca, altitude 2500 m., 10 December, 1894, *Rev. Lucius C. Smith, 367* (Gray Herb., photograph and fragments in Mo. Bot. Gard. Herb.), TYPE.

Although only the upper part of the plant is at present known to the writer, nevertheless it evidently belongs to the section *Suffruticosi* and appears to be most closely related to *S. Picridis* Schauer and *S. alvarezensis* Greenm. From the former it differs by the usually broader base of the upper stem leaves, more numerous heads and conspicuous bracteoles, while from the latter it is readily separated on foliar characters alone.

Senecio (§ **Palmatinervii**) **albonervius** Greenm. Monogr. Senecio, pt. 1, 26. 1901; in Engl. Bot. Jahrb. **32**: 22. 1902, nomen.

Arborescens 2–4 m. altus; caule tereti primo albo-tomentuloso maturitate glabrato et cortice brunneo tecto; foliis petiolatis basi palmatinerviis late ovatis 3–5 cm. longis latisque sinuato-5–11-lobatis remote calloso-mucro-denticulatis basi cordatis juventate utrinque tomentulosis plus minusve glabratis supra in nerviis persistenter albo-tomentulosis, petiolis plerumque 3–10 (usque ad 14 cm.) longis; inflorescentiis terminalibus paniculatis multicapitatis; capitulis 10–12 mm. altis radiatis; involucris anguste campanulatis vel subcylindricis brevicalyculatis; involucri squamis circiter 8 lineari-lanceolatis vel oblongis obtusis 5–6 mm. longis glabris vel parce tomentulosis; flosculis liguliferis plerumque 5, ligulis 5–7 mm. longis flavis 4-nervatis, pappi setis tubo corollæ longioribus; floribus disci 8–10; achæniis glabris.

Specimens examined:

Mexico: State of Mexico, Valley of Temascaltepec, April, 1831, *Schiede* (Berlin Herb. and Gray Herb.), TYPE; open woods, Ixtaccihuatl, altitude 2430–3350 m., March-July, 1903, *C. A. Purpus, 201* (Gray Herb. and Mo. Bot. Gard. Herb.). State of Vera Cruz, Mineral del Monte, *Ehrenberg, 324* (Berlin Herb. and Gray Herb.). State of Morelos, Sierra de Tres Marias, altitude 3050 m., 15 April, 1904, *C. G. Pringle, 8903* (Gray Herb. and Mo. Bot. Gard. Herb.). State of Michoacan, north slope of Mt. Tancitaro, altitude 2280–3200 m., 24 February, 1903, *E. W. Nelson, 6904* (U. S. Nat. Herb. and Gray Herb.).

The broadly ovate, shallowly sinuate-lobed leaves with persistent white tomentum on the veins of the upper leaf-surface, together with a terminal many-headed panicle and yellow ray-flowers, render this species distinct and easily recognized among all those of the palmately veined section to which it belongs.

Senecio angulifolius DC., var. **ingens,** var. nov.

Habitu et foliis formæ typicæ; inflorescentiis compactis pauci- vel multi-capitatis, bracteis bracteolisque perconspicuis; capitulis 1.5–2 cm. altis 40–45-floris radiatis vel discoideis.

Specimens examined:

Mexico: Mt. Ixtaccihuatl, above timber line, March-July, 1903, *C. A. Purpus, 193* (Mo. Bot. Gard. Herb.), TYPE; rocky slopes, Mt. Ixtaccihuatl, altitude 5790–6090 m.,

November, 1905, *C. A. Purpus, 1517* (Mo. Bot. Gard. Herb.). State of Puebla, Mt. Orizaba, near Chalchicomula, 25 February, 1892, *Jared G. Smith, 473* (Mo. Bot. Gard. Herb.).

On account of the conspicuous more or less foliaceous bracts of the inflorescence *S. angulifolius* DC. is a very characteristic species and is almost always recognized without difficulty. There is, however, a considerable variation in the size of the heads and in the number of flowers of the disk, as well as in the degree of development of the ray-flowers. In fact the latter may be well developed, more or less reduced, or entirely absent. The extremely large headed form, which is well exemplified by the specimens cited above, seems well worthy of varietal recognition. Doctor Purpus's No. 1517 is somewhat intermediate between the species and the variety.

Senecio (§ **Palmatinervii**) **brachyanthus** Greenm. Monogr. Senecio, pt. 1, 26. 1901; in Engl. Bot. Jahrb. **32**: 22. 1902, nomen.

Verisimiliter frutex; caule tereti cortice brunneo tecto juventate hirtello-puberulento glabrato; foliis longipetiolatis subpeltatis palmatinerviis suborbicularis circiter 7-lobatis membranaceis utrinque parce hirtellis subtus pallidioribus mucrodenticulatis, petiolis usque ad 13 cm. longis minute puberulentis; inflorescentiis terminalibus subglanduloso-hirtellis; capitulis subcylindricis 10–12 mm. altis heterogamis; involucri bracteis 8 lanceolatis 8–10 mm. longis acutis vel obtusis plus minusve purpurascentibus extus subglanduloso-hirtellis; flosculis femineis 5 multum reductis, ligula nulla, tubo gracili squamis involucri breviore; floribus disci 8–10; pappi setis albis; achæniis glabris.

Specimen examined:

Mexico: State of Guerrero, between Ayusinapa and Petatlan, altitude 1540–2155 m., *E. W. Nelson, 2137* (Gray Herb. and U. S. Nat. Herb.), TYPE.

The leaves and reduced ray-flowers of this species are similar to those of *S. cordovensis* Hemsl., but the character of the involucre indicates a closer relationship with *S. chapalensis* Watson.

Senecio (§ **Palmatinervii**) **chapalensis** Watson, Proc. Am. Acad. **25**: 155. 1890.

Var. **areolatus** Greenm. Monogr. Senecio, pt. 1, 26. 1901; in Engl. Bot. Jahrb. **32**: 22. 1902, nomen.

A forma typica recedit foliis utrinque glabratis subtus areolatis, petiolis usque ad 15 cm. longis plus minusve purpurascentibus; flosculis liguliferis granditer reductis.

Specimen examined:

Mexico: State of Morelos, on shaded bluffs of a wet canyon above Cuernavaca, altitude 1980 m., 15 February, 1899, *C. G. Pringle, 8010* (Gray Herb. and Mo. Bot. Gard. Herb.), TYPE.

Senecio (§ **Palmatinervii**) **Chrismarii** Greenm. Monogr. Senecio, pt. 1, 26. 1901; in Engl. Bot. Jahrb. **32**: 22. 1902, nomen.

Frutex; caule primo parce pubescenti maturitate glabro; foliis petiolatis palmatinerviis circumscriptione triangulari-ovatis 7–10 cm. longis 5–8 cm. latis hastatis 3–5-lobatis ciliatis mucro-denticulatisque granditer cordatis supra sparse hirtello-puberulentis subtus glabris vel in nervis puberulentis, lobiis mucronato-acutis; petiolis gracilibus 4–9 cm. longis parce hirtellis vel glabris; inflorescentiis terminalibus laxe paniculatis paucicapitatis dense glanduloso-puberulentis, pedunculis gracilibus remote bracteatis; capitulis 1.2–1.5 cm. altis discoideis paucicalyculatis; involucri squamis sæpius 8 lanceolato-oblongis ca. 1 cm. longis acutis penicillatis extrinsecus hirtello-puberulentis plus minusve purpurascentibus interioribus scarioso-marginatis; floribus disci plerumque 20 involucri bracteis longioribus; pappi setis albis; achæniis glabris.

Specimen examined:

Mexico: without definite locality, *Chrismar* (Berlin Herb., tracing and fragments in Gray Herb.), TYPE.

The affinity of this species is with *S. hederæfolius* Hemsl., *S. anisophyllus* Klatt, and *S. alienus* Robinson & Seaton. From the first two it differs in having deeply cordate leaves with more or less reflexed lateral lobes, and from the last it is readily separated by the deeply cordate leaves and absence of peltation.

Senecio (§ **Palmatinervii**) **hypomalacus** Greenm. Monogr. Senecio, pt. 1, 26. 1901; in Engl. Bot. Jahrb. **32**: 22. 1902, nomen.

Plate 10.

Frutex erectus; caule tereti primo dense sordido-puberulento, sæpissime lenticellis intermixtis, maturitate cortice brunneo tecto; foliis petiolatis vel supremis sessilibus circumscriptione ovato-rotundatis vel ovato-oblongis palmato-3–5-nerviis distincte 5–11-lobatis supra crebe crispo-hirtellis subtus lanato-tomentosis basi cordatis vel subtruncatis, margine sinuatis calloso-denticulatis ciliatis; petiolis usque ad 6 cm. longis; inflorescentiis terminalibus paniculatis polycephalis subglanduloso-hirtellis; capitulis 10–12 mm. altis parce calyculatis radiatis; bracteis involucri plerumque 8 (non-nunquam 7) oblongis vel subobovatis 5–6 mm. longis obtusis vel acutis extus crebe subglanduloso-hirtellis, interioribus late scarioso-marginatis; flosculis femineis liguliferis, ligulis anguste oblongis 5–6 mm. longis flavis; floribus disci circiter 10 (7–13) quam involucrum bis tanto fere longioribus; pappi setis albis; achæniis glabris.

Specimens examined:

Mexico: State of Oaxaca, mountains of Telixtlahuaca, altitude 2375 m., 10 December, 1894, *Rev. Lucius C. Smith, 368* (Gray Herb., photograph and fragments in Mo. Bot. Gard. Herb.), TYPE; Sierra de San Felipe, altitude 2130–2440 m., 17 November, 1894, *Charles L. Smith, 210* (Mo. Bot. Gard. Herb.); Cerro de San Felipe, altitude 1900 m., 25 September, 1895, *C. Conzatti, 119* (Gray Herb.).

This species is related to *S. oaxacanus* Hemsl., but differs from it in having distinctly lobed leaves which are thicker in texture, densely subglandular-hirtellous above and soft tomentose beneath; moreover, the leaf-margin of *S. hypomalacus* is markedly sinuate and the lobes show a tendency to become again lobate. C. and E. Seler's No. 1581 from Tillantongo, which has been referred to *S. oaxacanus* Hemsl., is somewhat intermediate between the two species, but it has the leaf-outline and thinner texture of Mr. Hemsley's species.

Senecio (§ **Palmatinervii**) **Kerberi** Greenm. Monogr. Senecio, pt. 1, 26. 1901; in Engl. Bot. Jahrb. **32**: 22. 1902, nomen.

Herbaceus robustus perennis usque ad 3m. altus; caule tereti erecto glabro vel parce tomentuloso; foliis petiolatis palmato-5–7-nerviis ovato-oblongis 5–10 cm. longis 5–8 cm. latis 5–7-lobatis carnoso-denticulatis reticulato-venosis supra sparse hirtellis subtus subarachnoideo-tomentulosis, lobis obtusis vel

subrotundatis et mucronato-acutis; petiolis 2–2.5 cm. longis; inflorescentiis terminalibus paniculatis multi-capitatis pubescentibus, pedunculis minute bracteatis; capitulis 7–8 mm. altis radiatis; involucris campanulatis minute calyculatis fere glabris; involucri squamis 13 lineari-lanceolatis vel lanceolato-oblongis 4.5–5 mm. longis acutis glabris; flosculis femineis 5 liguliferis, ligulis oblongis 4–5 mm. longis flavis; floribus disci ca. 14, pappi setis albis; achæniis glabris.

Specimen examined:

Mexico: "Tromptero, Mesa del Arrero," 21 November, 1880, *Kerber, 94* (Berlin Herb., fragments and tracing in Gray Herb.), TYPE.

This species is known at present from a single specimen in the Royal Botanical Museum of Berlin. From this specimen the writer was permitted, as in a number of other cases, while making a study of the genus several years ago, to make a tracing and take fragments for the Gray Herbarium of Harvard University. The species is related to *S. Hartwegi* Benth. and *S. reglensis* Greenm., but from these and from other species of the section *Palmatinervii* to which it belongs, it is readily distinguished by the somewhat elongated more or less fan-shaped and bluntly lobed leaves.

Senecio (§ **Palmatinervii**) **velatus,** sp. nov. Plate 13.

Frutex; caule tereti carnoso ramoso ad apicem sordido-tomentoso cetero glabro in sicco cortice brunneo tecto; foliis petiolatis palmato-7-nerviis circumscriptione ovato-rotundatis ca. 10 cm. longis latisque angulato-7–9-lobatis membranaceis integris juventate utrinque plus minusve albo-tomentosis subtus persistenter arachnoideo-tomentulosis, lobis triangulari-ovatis mucronato–acutis; petiolis ca. 8 cm. longis floccoso-pubescentibus; inflorescentiis terminalibus dense cymoso-corymbosis minute bracteatis multicapitatis glabris vel in axillis ramulorum floccoso-tomentulosis; capitulis ca. 1.5 cm. altis radiatis; involucri subcylindrici squamis sæpius 8 lanceolato-linearis vel lanceolato-oblongis 7–10 mm. longis acutis vel obtusis; flosculis liguliferis 3–5, ligulis anguste oblongis ca. 1 cm. longis; floribus disci 6–7, pappi setis albis; achæniis glabris striatis.

Specimen examined:

Mexico: State of Jalisco, on bluffs of barranca, near Guadalajara, 20 May, 1891, *C. G. Pringle, 5160* (Gray Herb., photograph and fragments in Mo. Bot. Gard. Herb.), TYPE.

The writer has withheld publication of this species for several years with the hope that additional material might be secured. Mr. Pringle's specimen, from which the above description is drawn, is in the Gray Herbarium and consists of a terminal portion of a flowering stem and two detached leaves. In stem and inflorescence characters it corresponds very well with typical specimens of *S. præcox* DC. except that the terminal portion of the stem and branches are covered with a tawny pubescence, not glabrous as is usually the case with the DeCandollean species. On account of the similarity of stem and inflorescence and because of the detached leaves the plant has been referred doubtfully to the peculiarly characteristic and well known *S. præcox* DC.

The extreme care with which Mr. Pringle prepared his plant material and the fact that the leaves on the specimen under consideration, although detached from the stem, accord with the type of foliage of the section *Palmatinervii* lead me to believe that we have to deal in the present case with an unrecorded species related to but distinct from *S. præcox* DC., and in all probability one of limited geographical distribution.

Senecio Klattii, nom. nov.

S. roseus Klatt, Ann. k. k. Naturhist. Hofmus. Wien **9**: 366. 1894, not. *S. roseus* Schz. Bip. in Flora **28**: 498. 1845.

Explanation of Plate

PLATE 10

Senecio hypomalacus Greenm.

Mexico

From the type specimen, Rev. Lucius C. Smith No. 368, in the Gray Herbarium of Harvard University.

GREENMAN—NORTH AMERICAN SENECIONEAE

COCKAYNE, BOSTON

Explanation of Plate

PLATE 11

Senecio oreopolus Greenm.

California

From the type specimen, Hall and Babcock No. 5526, in the Gray Herbarium of Harvard University.

GREENMAN — NORTH AMERICAN SENECIONEAE

COCKAYNE, BOSTON

Explanation of Plate

PLATE 12

Senecio iodanthus Greenm.

Mexico

From the type specimen, Pringle No. 4302, in the Gray Herbarium of Harvard University.

GREENMAN — NORTH AMERICAN SENECIONEAE

COCKAYNE, BOSTON

Explanation of Plate

PLATE 13

Senecio velatus Greenm.

Mexico

From the type specimen, Pringle No. 5160, in the Gray Herbarium of Harvard University.

GREENMAN — NORTH AMERICAN SENECIONEAE

COCKAYNE, BOSTON

Explanation of Plate

PLATE 14

Senecio subauriculatus Greenm.
Mexico

From the type specimen, E. W. Nelson No. 2526, in the Gray Herbarium of Harvard University.

GREENMAN — NORTH AMERICAN SENECIONEAE

COCKAYNE, BOSTON

A STUDY OF THE PHYSIOLOGICAL RELATIONS OF SCLEROTINIA CINEREA (BON.) SCHRÖTER

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Introduction

This paper reports the results of an experimental study regarding certain physiological activities of the brown-rot fungus of stone fruits. The investigation concerns itself primarily with the conditions influencing the penetration and infection of green and ripe fruits by the fungus in question, the action of the parasite on the host cell, and the secretion of the enzymes which act upon the cellulose and pectic substances of the host. The work was undertaken with the hope of throwing some further light upon the factors concerned in fungous parasitism. Our present conception of this subject is based upon fragmentary and, in some respects, contradictory evidence. However, each year there are acquired new facts, or new applications of known facts, bearing upon this exceedingly involved and complex question. An examination into the history of investigations concerning the interaction of host and parasite shows that the study of this subject dates back to the work of the pioneers in plant pathology; modern methods and recent discoveries have, however, given an added impetus to research along this line.

Progress in combating fungous diseases depends not only upon a familiarity with the life history of the parasite, but more especially upon an intimate knowledge of the metabolism of the parasite and the nature of the changes which it induces in the host. Indeed, many of our recommendations for controlling parasitic diseases of plants will perhaps be modified when a more exact knowledge of the interrelations of host and parasite is gained. Furthermore, a more intimate knowledge of the physiological aspects of plant pathology will undoubtedly throw much light on the question of immunity and susceptibility.

We should, of course, like to know more about the factors favoring or inhibiting parasitic action, as well as the conditions

which influence the infection and the penetration of parasitic fungi. It would also be interesting to know why some fungi are so virulent and rapid in their destructive action on the host; for instance, it would be instructive to know whether it is due to the secretion of an enzyme, or a toxic substance (e. g., some acid), or to the disturbance of the osmotic relations of the host cells, or to some other perhaps unknown factor. For a study of some of these problems the writer has chosen as the organism *Sclerotinia cinerea* (Bon.) Schröter, the fungus causing the brown rot of stone fruits. This form is particularly suitable for the purpose since it is a virulent parasite, yet grows well as a saprophyte —readily lending itself to cultivation in the laboratory.

Historical Review

Space will permit only a brief review of some of the more important papers dealing with certain aspects of this subject. Much of the literature that is indirectly concerned with the problem, or that is fully reviewed or superseded by subsequent publications, will not be discussed here.

In the period from 1858 to 1878 little experimental evidence appeared concerning the nature of the action of fungous parasites, although several writers make mention of the penetration of host cells by fungous hyphae. Penetration was then frequently spoken of as merely a process of boring through ("durchbohrung") the host tissue, Kühn (34), as early as 1858, mentioning this fact in a discussion of the potato-blight fungus. A few years later, in 1863, de Bary (1) speaks of the penetration of the host by *Peronospora*, and further makes mention of this fact in connection with his work on the rusts (2); again in his work 'Morphologie und Physiologie der Pilze, Flechten, und Myxomyceten' (3) he discusses the penetration of the host, but says he has no knowledge of the force that causes this boring into the host tissue.

Hartig (26), in his early work on wood-destroying fungi, as well as in his later investigations, emphasizes the fact that fungi are able to destroy cellulose. By a microscopical study of diseased wood he found that the properties of the latter are very materially changed by the fungus; he did not, however, attempt to isolate an enzyme.

De Bary (4), in 1886, gives us the first important contribution to our knowledge concerning the action of parasites on host cells. This author, in his epoch-making research on the fungus now known as *Sclerotinia libertiana*, reports that the organism secretes a substance that discolors, plasmolyzes, and finally kills the host cells. This toxic secretion penetrates the host cells in advance of the fungus, killing them before they are actually pierced by the fungous filaments. De Bary was able to isolate this toxic substance, which he considered as probably an enzyme, and found that it would cause an injury to the host tissue similar to that produced by an attack of the fungus itself. He holds that the fungus will not grow on living tissues, for it attacks only through a wound and kills the cells in advance of itself, thus not actually growing upon the living tissue. The product resulting from the disintegration of the cell wall of the host was thought to be a sugar that served as food for the fungus. In this connection de Bary also mentions finding oxalic acid encrusting the older fungous filaments.

The next important paper on the interaction of host and parasite was that of Marshall Ward (51) published just two years after de Bary's work and concerning itself with a species of *Botrytis* causing a lily disease. In this excellent piece of work the author showed that the fungous hyphae on coming in contact with such solid substances as sections of a lily bulb, or even a cover glass, secrete from the tips drops of a substance that has a very peculiar effect on the host cell. He found that a water extract of this secretion when applied to sections of a lily bulb will cause the cell walls to swell and to assume an abnormal appearance; the middle lamella is first dissolved and finally the entire cell wall is disorganized. Ward does not consider that this toxic secretion is stimulated by starvation.

Several investigators have held that the penetration of many fungi is due to chemotropism, i. e., that penetration of the fungous hyphae is due to some stimulus which the constituents diffusing slowly from within the host cells exert. Büsgen (16), Miyoshi (39), Behrens (6), Schmidt (44), and others have adhered to the view that chemotropism is important, but more recent work, such as that of Fulton (25), does not uphold the theory.

Behrens (6) investigated some of the physiological relations of saprophytes in comparison with parasites, using *Mucor stolonifer*, *Penicillium sp.*, *Botrytis cinerea*, and *Oidium* (=*Sclerotinia*[1]) *fructigenum*. This author holds that *Sclerotinia* does not produce a cellulose-dissolving enzyme, and that the fungus merely forces its way through the host tissue by a purely mechanical force, or that, in some cases, it splits the middle lamella but does not dissolve it. In the case of the other fungi mentioned above he believes that an enzyme is secreted which dissolves the middle lamella. The cause of the injury due to *Sclerotinia*, he holds, is not that the cellulose walls or the pectin of the middle lamella is dissolved, but that the turgor and the osmotic relations of the penetrated cells are materially modified. According to this author some substance diffuses through the walls and stimulates the fungus to bore through or between the cell walls. He demonstrated in *Botrytis* and *Penicillium*, moreover, a thermo-stable toxic body which disintegrated the host cells, and believes that these fungi secrete a pectin-dissolving enzyme which is different from that which acts upon cellulose.

Nordhausen (40), at about the same time, made similar studies on *Botrytis cinerea* and comes to similar conclusions. He finds that the enzyme does not cause a strong swelling of either the middle lamella or the cellulose cell walls, the action in this respect being more like that of de Bary's *Sclerotinia*. Smith (46) studied the parasitism of *Botrytis cinerea*, but in certain particulars did not get the same results as de Bary and Ward. Like them he finds that the parasite secretes some soluble substance that penetrates and kills the living cells in advance of the fungous filaments, but unlike Ward he could detect no swelling of the cell wall. Smith believes that this toxic substance is not an enzyme, for boiling does not inactivate it, but thinks that it is perhaps oxalic acid, since this substance is always present in the cultures and amounts in some cases to as much as two per cent. The analytical methods whereby the oxalic acid was determined, unfortunately, are not given.

Schellenberg (43) investigated the action of several saprophytic and parasitic fungi on hemicelluloses from a number of

[1] Wehmer, C. Ber. d. deut. bot. Ges. **16**: 298–307. 1898; Saccardo, Syll. Fung. **4**: 34. 1886.

different sources. He claims that these fungi act differently toward different celluloses, dissolving some and having no effect on others. The nature of the penetration and the action of certain parasites on the host tissue were also studied. There was no case in which *Botrytis* dissolved true cellulose, but it readily dissolved the hemicellulose part of the cell, leaving the cellulose intact. According to this author, therefore, the penetration and dissolving action of such parasites as *Botrytis vulgaris* is due to their ability to dissolve hemicelluloses. He considers that the middle lamella is largely composed of hemicelluloses or closely allied substances. According to this view, therefore, organisms that dissolve the middle lamella are essentially hemicellulose-dissolving forms. As a result of his studies on *Sclerotinia fructigena* and *S. cinerea*, Schellenberg finds a different action on different fruits, but in no case does he report a splitting of the cells along the line of the middle lamella, as some previous investigators have reported. He believes that there is a slight dissolving action on that part of the cell wall which is in immediate contact with the fungous filament, but that the rest of the cell wall remains intact. In the twigs also he finds that the fungus dissolves the hemicellulose and leaves the true cellulose unacted upon.

An extensive literature has developed concerning the enzymes of importance in the nutrition of fungi, but since these investigations either deal with saprophytes, or are only indirectly concerned with the work to be reported in this paper, it will be unnecessary to do more than mention some of the papers here. Among the more important contributors may be mentioned Ward (50, 52), who was the first to use pure cultures of a wood-destroying fungus (*Stereum*), Biffen (9), who studied the biology of *Bulgaria polymorpha*, Bourquelot and Hérissey (13), who investigated the enzymes in sporophores of *Polyporus sulphureus*, Czapek (18), who made his investigations with natural infections of *Merulius lacrymans* and with other fungi, Kohnstamm (33), who worked on some species of *Merulius*, Buller (14, 15), who investigated sporophores of *Polyporus squamosus*, Van Iterson (28), who developed methods for isolating cellulose-dissolving bacteria and fungi, and Dox (19), who investigated the enzyme action of species of *Penicillium* and *Aspergillus*. It is interest-

ing to note that although we have every reason to believe that cytase is present in timber-decay organisms yet its presence has been demonstrated only indirectly by cytological methods. It is true, however, that many of the investigators mentioned above who found no cytase used the sporophores in their experiments and not the mycelium.

The status of the subject of the enzymes concerned in the metabolism of parasitic fungi is given in Reed's recent publication (42), which concerns itself with the enzymes produced by the parasitic fungus *Glomerella rufomaculans*. This author has proved that the parasite produces many of the enzymes that had previously been reported for saprophytes, and by quantitative methods has demonstrated different enzymes acting on the several classes of nutritive substances, such as carbohydrates, glucosides, fats, and proteins. He did not, however, investigate the cytolytic activity of the fungus but states that the nature of the diseased host would indicate that cytase very probably is not produced by this fungus. Peltier (41), as a result of his investigations with *Botrytis Fuckeliana*, finds that the host cells are killed in advance of the fungous penetration, and that the parasite secretes a thermo-stable toxic substance, but, unlike Smith, finds no oxalic acid. The method of testing for oxalic acid unfortunately is not given.

The action of bacteria on cellulose and other plant products has been extensively studied by a number of investigators, but for the purpose at hand it will suffice to cite some of the more recent publications in which the earlier literature is reviewed. The work of Jones (29, 30), which gives a good resumé of the early work on this subject, is reviewed below under the discussion of pectin.

McBeth and Scales (38) report that a number of bacteria and fungi hydrolyze cellulose and claim that filamentous fungi play a very important rôle in the destruction of cellulose in soils. The cellulose-destroying fungi, according to these authors, act differently toward different kinds of cellulose, but their experiments do not seem to support this conclusion. Kellerman and McBeth (32) have also contributed to our knowledge of the cytolytic activity of fungi. Kellerman (31) has employed a method

by which it is demonstrated that cytase diffuses in agar considerably beyond the region of hyphal penetration, and that a portion of the agar containing the enzyme dissolves cellulose in a manner similar to that of the fungus itself.

The organism employed in my work was isolated from an infected plum twig, at Madison, Wisconsin. The original cultures were taken from a single colony in a Petri dish, this procedure giving reasonable assurance that I was working with a single strain of the organism. Regarding the systematic relations of this organism a word may not be out of place here, since considerable confusion has arisen in the literature regarding the specific name of the organism causing the brown rot of stone fruits (27, 53, 37). Woronin (56) has made an important contribution designed to establish the systematic position of the two species *Sclerotinia cinerea* and *S. fructigena*. It has generally been held that *S. fructigena* causes the brown rot of stone fruits in this country, while in Europe this fungus is found only on pome fruits; but Matheny (37) has recently given good evidence tending to show that it is *S. cinerea* which causes the brown rot of stone fruits both in this country and in Europe.

Experimental Studies

Infection

Some investigators, as, for instance, Zschokke (57), have held that *Sclerotinia cinerea* is unable to penetrate sound fruit, while Smith (45), among others, has held that the fungus rapidly penetrates and infects sound and unwounded fruit (peaches). Casual observation in the field would seem to justify the former view, for those fruits in contact with other fruits or twigs, and therefore liable to puncture or abrasion, are the ones that are usually found infected; indeed, field observations and laboratory experiments point to the conclusion that infection takes place much more readily, especially with immature fruits, when the cuticle is broken. One would, therefore, naturally raise the question as to whether or not infection can take place when the cuticle is unbroken, and if so under what conditions and in what stages of the development of the fruit. During the summer of 1913

the writer performed a number of experiments which throw more light on the question of the infection of the host.

Methods and Results.—The methods employed were as follows: Plum twigs bearing leaves and fruit were broken off and brought into the laboratory, washed with a mercuric chloride solution (1–1000) and in sterile water. They were then suspended in sterile moist chambers prepared by placing moistened absorbent cotton in the bottom of wide-mouthed one-liter Erlenmeyer flasks that had previously been plugged and sterilized. Twigs having one or more green leaves were used in every case, for in this way green plums hang on the twigs and remain alive for some time. This method was especially applicable here, for it enabled one to maintain absolutely sterile conditions in a moist atmosphere and at the same time keep the host living and in a normal condition. The results of these infection experiments are given in table 1.

Discussion of Results.—From these results it is evident that plums were infected as early as June 27, at which time they were immature, in fact not more than half-grown. Infection did not take place when a spore suspension was placed on very green and immature plums unless the epidermis was broken or punctured. There were, however, some instances where plums remained healthy in the flask for two or three weeks and became infected only after the lapse of time had brought about an artificial maturity. On the other hand, plums that were approaching maturity, though not mature, as well as mature fruits, may be infected by applying a spore suspension to the natural surface, i. e., a surface which has not been punctured or injured in any way. In this connection it should be mentioned that infection was much more readily accomplished when two plums were hanging so as to be in contact with each other than when they were not touching. This, no doubt, was due to the fact that a drop of water containing spores may be held between the plums long enough for spore germination and infection to take place. These results also indicate that infection takes place readily without puncturing when a portion of the mycelial felt is laid on the surface of either green or ripe fruit.

It should be noted here that one can sometimes find plums in the field only half-grown which are affected with the brown-rot

TABLE I

RESULTS OF INFECTION EXPERIMENTS WITH SCLEROTINIA CINEREA

Date	Fruit	Inoculating material	Treatment of surface	Method of inoculation	Results
June 27	Green plums	Spore suspension	Cuticle killed by steam	Surface application	++*
June 27	Green plums	Spores	Skin punctured with needle	Needle puncture	+
July 2	Green plums	Spores	Skin punctured with needle	Needle puncture	++
July 8	Green plums	Spores	Skin punctured with needle	Needle puncture	++
July 8	Green plums	Spore suspension	Untreated	Surface application	−
July 8	Sour cherries	Spores	Skin punctured with needle	Needle puncture	+
July 23	Green plums	Spores	Skin punctured with needle	Needle puncture	++
July 23	Green plums	Spore suspension	Untreated	Surface application	−
July 23	Green plums	Spore suspension	Skin cut	Surface application	++
July 23	Ripe plums	Mycelium	Untreated	Surface application	++
July 23	Green plums	Mycelium	Untreated	Surface application	++
July 30	Green plums	Mycelium	Untreated	Surface application	++
Aug. 5	Green plums	Spore suspension	Untreated	Surface application	+
Aug. 13	Nearly ripe plums	Spore suspension	Untreated	Surface application	++
Aug. 13	Ripe plums	Spore suspension	Untreated	Surface application	++

*++ indicates that practically every inoculated fruit became infected.
+indicates that only a portion of the inoculated fruits became infected.
− indicates that none of the inoculated fruits became infected.

fungus, but so far as the writer's observation indicates, infection in these cases takes place through the twig, or, in some cases, through another plum with which it is in contact and which in turn is infected through the twig. Nevertheless, field observations also verify the laboratory work in that plums (especially certain varieties, such as Wood) when approaching maturity may be infected in the field without being in contact with other fruits and without having any visible punctures or wounds in the skin. All these experiments and observations point to the conclusion that penetration of the cuticle is a very important factor in the infection of fruits, especially immature fruits; that infection of very green fruits without punctures is rare; and, on the other hand, that maturing fruits without punctures may be readily infected both by spores and by a mycelial felt in the field and in the laboratory.

PENETRATION

The nature of penetration and the course of the hyphæ of parasitic fungi in piercing host tissue is an interesting and important question in connection with a study of the nature of parasitic action. In the case of the brown-rot fungus growing on the plum it is of importance to know whether or not the hyphæ merely follow the middle lamellæ or whether they enter the cells wherever they come in contact with them. Previous investigators differ very widely in their opinions as to the nature and course of the penetration of the fungus in question, a condition which is perhaps partly explained by the fact that different hosts were employed in the various investigations. Furthermore, it appears that the methods employed in some of the researches were not of such a character as to readily yield complete information concerning all the facts in the case.

In my own work a study of the penetration of the host tissue by the fungus was made by examining a number of sections of infected tissue in which the disease had reached various stages of development, and comparing them with sections of healthy tissue from the same fruit. For this purpose a special method was employed.

Methods and Results.—Small pieces of fruit composed of diseased and sound tissue were cut from plums inoculated with

a pure culture of the fungus. These segments were immersed in 70 per cent alcohol just long enough to partially kill the fungous filaments and the host cells, yet not long enough to discolor the sound tissue or to modify or change the color of the diseased tissue in any way. From this material razor sections, containing both diseased and healthy tissue, were made, stained for a short time in eosin, and then partially destained with alcohol. If the pieces of plum had not remained in the alcohol for a sufficient length of time, the razor sections were immersed in 70 or 95 per cent alcohol before staining. By employing this method it is possible to stain the fungous filaments deeply, while the host tissue remains unaffected. Indeed, this method permits of a rather sharp color differentiation between the healthy and the diseased tissue, the latter being blackened by the disease. This method, though quite applicable for the purpose at hand, was primarily developed for another purpose, which will be discussed below.

Since every fungous filament is very sharply differentiated, one may readily study the course of the hyphæ with reference to the host cells. By staining, sectioning, and examining diseased material taken from the margin of the infected area, one finds the fungous hyphæ penetrating the cells at any point of contact; indeed, after examining a number of specimens by the method reported above, the writer finds no indications that the fungous hyphæ follow the middle lamellæ, as has been reported by other investigators (57, 6) for pears and other fruits. The above method also enables one to contrast the cell walls of infected and penetrated cells with those of normal tissue. It is entirely possible that the fungous filaments, on coming in contact with a cell wall, secrete just enough enzyme to dissolve their way through the cell walls, leaving the walls of the host cells surrounding the hyphæ entirely normal, i. e., without swelling or disorganization.

Another and somewhat different experiment was performed to get additional evidence on this point. From sound plums which had previously been rendered sterile by washing in bichloride of mercury solution (1–1000) and sterile distilled water, free-hand sections were cut with a razor sterilized in 50 per cent alcohol. The sections were arranged in hanging drop cultures

and each inoculated with a drop of a very dilute spore suspension containing two or three spores per drop. The progress of the fungus and the condition of the host cells were noted from day to day but no visible disintegration of the cell walls could be observed, nor did the fungus show any particular affinity for the middle lamellæ.

Conclusions.—We would conclude, therefore, as a result of direct observation on the host tissue, that the fungus penetrates the host very readily and rapidly, that it does not necessarily follow the middle lamellæ in the plum and the peach, and that there is no visible general disintegrating action on the middle lamellæ or on the cell walls of the living host.

ACTION OF THE FUNGUS ON THE LIVING HOST CELLS

A significant fact in the metabolism of the brown-rot fungus is that it induces such an exceedingly rapid decay in the infected fruits. This rapid decay might be connected both with a rapid growth of the fungus and with a pronounced power which the organism possesses of breaking down and changing the constituents of the host. Moreover, several representatives of the genus *Sclerotinia* have been reported to have the power of secreting an enzyme or some other substance which kills the host cells in advance of penetration. Were this the case, it would be expected that rapid decay would accompany the action of the parasite. Is this view applicable to the action of *Sclerotinia cinerea?* The investigators who have made a study of this organism differ very widely in their views regarding the effect which it has on the host tissues, and it seemed desirable, therefore, to determine the relation of hyphal penetration to the death of the cells.

Methods and Results.—In order to fix the material for this study, it was found satisfactory to proceed as follows: Small pieces of the host tissue were taken from the margin of the diseased area and placed in 95 per cent alcohol for a short time. Free-hand sections were made of this material so as to include both diseased and healthy cells, and the sections stained for a short time in eosin and subsequently decolorized in part with alcohol, if necessary to give the desired contrast. By this

method the fungus may be distinctly differentiated from the host tissue, the killing and staining agents having little or no effect on the host cells. There is a more or less sharply differentiated line of demarcation between the injured and the sound cells, as indicated by the darker color of the former. The effect of the fungus is readily discerned by the blackening of the host tissue, this being especially noticeable in green plums. The discolored and poisoned cells are not at first plasmolyzed, and it is to be noted here that discoloration rather than plasmolysis should be taken as the index of the toxic action of this fungus on its host. It should perhaps be mentioned here, too, that the blackened cells shade off somewhat gradually into the hyaline healthy ones, and that, therefore, there is not always a sharp line of demarcation between the diseased and the healthy cells. However, in spite of these difficulties, I was convinced, after having examined a large number of sections of diseased and healthy tissue, that there is no positive evidence that the host cells are discolored, and therefore injured and poisoned, in advance of actual penetration by the fungus.

The indirect method employed to determine the same point consisted in applying to sound fruits an extract from decayed plums. Fruits were disinfected with mercuric chloride solution, washed in sterile distilled water, and inoculated with *Sclerotinia cinerea*. When the plums had become thoroughly decayed the juice was extracted and filtered under sterile conditions through a Chamberlain filter. The juice thus obtained was incubated for one week at a temperature of 22–25° C., and also tested on nutrient agar plates, and found to be sterile by both methods. From sound plums, which had been disinfected in the usual manner, a cone-shaped plug was cut out and the resulting cavity filled with this sterile extract,—the controls being prepared in a similar manner, using sterile water instead of the plum extract. The results were negative, that is, the controls were not unlike those treated with the extract from decayed plums.

The same experiment was repeated in a modified form by using thin razor sections of both green and ripe plums, the sections being made under sterile conditions as before, and observed in a hanging drop of sterile juice from decayed plums.

By means of this method one could readily observe any changes that might take place in the cells and make accurate comparisons with controls. Frequent observations were made, and throughout this experiment, which continued for several days, one could not distinguish between the appearance of those sections in a drop of sterile water and those in the sterile extract from decayed plums. It is possible and perhaps probable that this fluid, being merely the juice of the fruit, was too dilute to be effective, but the experiment was made because of the possibility of positive evidence.

Discussion of Results.—The initial stage in the injury caused by this fungus is shown by discoloration only and not by plasmolysis, and therefore one cannot draw conclusions with absolute certainty as to the poisoning effect of the extract on the cells of a cut surface, for the latter turn brown as soon as exposed to the air, just as when infected with the organism. It was comparatively easy, however, to observe that the extract had no effect on the cell walls, for no difference could be observed between the cell walls of the tissue thus treated and those of the control specimens. Even where the sections were left in the extract for several days neither swelling nor disorganization of the cell walls or middle lamellæ was noted. When sections of plum tissue were inoculated with one or more spores of the brown-rot fungus no cell-wall disintegration resulting from the growth of the fungus could be observed. A comparative study of sections of tissue, respectively exposed and not exposed to the action of the extract from decayed fruit, showed that no difference could be detected between the two, and that, therefore, no enzyme with a perceptible cytolytic action exists under these conditions. It has been held by some, notably by Behrens (6), that the injury to the host cell is largely physical in that the fungus penetrates at such a prodigious rate that the fluids of the host cell are allowed to escape with loss of turgor to the latter; furthermore, that the osmotic equilibrium is soon destroyed, with plasmolysis and death ensuing. It is very probable that part of the rapid injury to the host can be explained on purely physical grounds, but this may not be the only factor involved, although we do not now know what chemical activity of the fungous cells may be concerned in the rapid killing of the host tissue.

ACTION OF THE FUNGUS ON CELLULOSE

A number of investigators have regarded cellulose dissolution as a very important factor in the parasitism of many fungi; indeed, some of the earlier workers seemed to consider this the prime factor involved. While it is a well known fact that there are many fungi, especially saprophytes, which hydrolyze, or dissolve, certain celluloses, research extending over a wide field has revealed the nature of parasitism to be a very complex one in which other factors are as important as the dissolution of cellulose and the cell wall.

It has been the writer's purpose to study from two different points of view the action of the brown-rot organism on celluloses, (1) by observing the action of the fungus on pure cellulose isolated from the host tissue, and (2) by studying microscopically its action on the host cell walls themselves. In the former study cellulose agar was used, the cellulose being isolated from plums by the methods discussed below.

Methods and Results.—In the above mentioned study of the action of the fungus on pure cellulose, a variety of reagents, media, and methods for the preparation of cellulose were employed, a brief account of which follows. Schweizer's reagent was prepared by adding a slight excess (40 grams to the liter) of copper carbonate to dilute ammonium hydroxide solution composed of three parts of water to ten parts of ammonium hydroxide (sp. gr. 0.90). The copper solution was then shaken vigorously, allowed to stand over night, and the supernatant solution siphoned off. This is the procedure employed by McBeth and Scales (38).

Paper cellulose from filter paper was prepared according to the method given by McBeth and Scales (38) by dissolving 15 grams of sheet filter paper in Schweizer's reagent, diluting about ten times with water, and precipitating the cellulose with a solution of one part of hydrochloric acid to five parts of water. This mixture was then further diluted to 15 or 20 liters, the supernatant liquid siphoned off, and the residue washed repeatedly with water until the precipitated cellulose was free from both copper and chlorine. After standing quietly for several days the clear liquid was siphoned off and the precipitate used for the preparation of cellulose agar.

Cellulose agar was made by adding about one per cent (estimated by the weight of the paper before treating with Schweizer's reagent) of precipitated paper cellulose, prepared as stated above, to a mineral nutrient solution, the complete medium having the following composition:

Cellulose suspension	500 cc.
Agar	10 grams.
Monopotassium phosphate, 1 gram Magnesium sulphate, 1 gram Sodium chloride, 1 gram Ammonium sulphate, 1 gram Calcium carbonate, 2 grams Tap water, 1000 cc.	500 cc.

The insoluble precipitate appearing in the mineral nutrient solution was filtered off before the cellulose suspension and agar were added. Good results were also obtained by using 0.5 gram of calcium nitrate instead of 2 grams of calcium carbonate, in which case filtering is unnecessary. The mineral nutrient solution having the composition tabulated above will be referred to as nutrient "A."

Another nutrient solution very low in organic matter was also employed in the cellulose agar, but with rather unsatisfactory results. This solution, which will be referred to as nutrient "B," is that employed by Reed (42), and is made up as follows, the only organic material present being the small amount of sodium citrate:

Ammonium nitrate	10 grams
Dipotassium phosphate	5 grams
Magnesium sulphate	1 gram
Sodium citrate	1 gram
Tap water	1000 cc.

In making the cellulose agar this nutrient solution was used in exactly the same way as nutrient "A."

Since previous investigators have held that the celluloses from various sources differ in their resistance to hydrolyzing enzymes, an attempt was made in this investigation to prepare a cellulose from a natural host—plums—of the parasite. In order to secure a cellulose that is modified as little as possible in the process

of isolation three different methods were employed in preparing cellulose from plums, the resulting products being designated, for convenience in reference, respectively as soda cellulose, washed cellulose, and potassium chlorate cellulose.

In the preparation of soda cellulose ripe plums were squeezed through cheese cloth and the pulp was washed thoroughly with water. The pulp was then treated with an 8 per cent solution of sodium hydroxide and heated in the autoclave at ten pounds pressure. After thoroughly washing the pulp with water the heating with alkali was repeated and the product given final washings until free from alkali.

The second method of isolating cellulose—washed cellulose—consisted in washing the fruit pulp with water until free from substances soluble in cold water. Water was then added and the mixture heated in the autoclave at 15 pounds pressure, and washed. The operation was repeated as long as any water-soluble substances could be detected. This method, of course, gives an impure cellulose, yet the product is one that is free from water-soluble substances.

The third method consisted in oxidizing, dissolving, and washing out the plum pulp until a pure cellulose—potassium chlorate cellulose—was obtained. Pulp, secured from ripe plums in the manner stated above, was washed with cold water until the wash water was free from solutes, and then treated with a cold solution composed of 30 grams of potassium chlorate dissolved in 520 cc. of cold nitric acid (sp. gr. 1.1). This mixture was kept in the ice box for about three weeks, at the end of which time the pulp was entirely white. This method[1] is said to yield a product that differs only very slightly from the original cellulose.

The product obtained by these various methods was not allowed to dry, for it is possible that drying changes the nature of cellulose so that it is more resistant to the action of cytolytic enzymes. A part of the cellulose obtained by each of the preceding methods was treated with Schweizer's reagent and precipitated with hydrochloric acid and washed as stated above under the preparation of filter-paper cellulose. These three cellulose preparations thus treated with Schweizer's reagent, as

[1] Fowler, G. J. Bacterial and enzymatic chemistry. 159. 1911.

well as the three corresponding untreated portions, were used in the preparation of cellulose agars, according to the method given above. The media were placed in test tubes of very small (8 mm.) diameter, and sterilized. The tubes of melted agar were then cooled rapidly in cold water in order to bring about the hardening of the agar before the cellulose had had time to settle to the bottom of the tubes.

Tubes of the various cellulose agars were inoculated with *Sclerotinia cinerea* and others with a species of *Penicillium*, which will be designated as *P. expansum*[1], isolated from decaying peaches and apples. Since these two fungi, viz., *Sclerotinia cinerea* and *Penicillium expansum*, act very differently toward the host, a word contrasting their action may not be out of place here. As a result of inoculating apples, peaches, or pears with a pure culture of *Sclerotinia* the host tissues are promptly killed, while the fruits remain practically as firm after complete decay as before inoculation. On the other hand, the fruits inoculated with the *Penicillium* become very soft and watery, developing a pustule or sunken area where the infection took place. One may assume, therefore, that the *Sclerotinia* does not materially affect the celluloses and pectic substances that make for the firmness of the fruit, while, on the other hand, *Penicillium* does affect these substances, causing the fruit to lose its firm consistency. Since these two fungi show such entirely different and opposing characteristics as regards their effect on the same host, it is interesting to compare their action in pure cultures on cellulose and pectin-like substances. Such a comparative study was made, the results of which are given in table II.

Discussion of Results.—The results given in table II indicate that both *Sclerotinia cinerea* and *Penicillium expansum* exhibited in general a very slight hydrolytic action when grown on cellulose isolated from the plum, there being very slight action with both fungi on the soda cellulose and also on the potassium chlorate cellulose and no action on the washed plum cellulose. On the other hand, both fungi very readily dissolve filter-paper

[1] A culture of this organism was sent to Dr. Chas. Thom, who very kindly examined it and gave as his opinion that it was *P. expansum*, or perhaps a strain of that species. The organism in question, when grown on the media employed by Thom, showed characters very similar to those of *P. expansum*, as given by Thom (48).

TABLE II

ACTION OF SCLEROTINIA CINEREA AND PENICILLIUM EXPANSUM ON CELLULOSE

Type of cellulose used	Nutrient solution added	Sclerotinia cinerea		Penicillium expansum	
		Growth	Cellulose hydrolysis	Growth	Cellulose hydrolysis
Soda cellulose	A	++†	–†	++	+
Soda cellulose	B	++	–		
Potassium chlorate cellulose	A	++	+	++	+
Potassium chlorate cellulose	B	++	+		
Washed ligno-cellulose*	A	+	–		
Washed ligno-cellulose*	B	—	–		
Washed cellulose	A	+	–	+	–
Soda cellulose (Schweizer's)	B	+	–	+	–
Soda cellulose (Schweizer's)	A	++	+		
Washed cellulose (Schweizer's)	A	+	–		
Soda cellulose	Peach juice	+++	–	+++	–
Filter paper strips	Peach juice	+++	–	+++	–
Filter paper strips	A	++	–	++	–
Filter paper strips	B	+	–	++	–
Filter paper strips	0.5% glucose solution	+++	–	+++	–
Filter-paper cellulose	A	++	+++	++	+++

*Ligno-cellulose is the name here given to cellulose from the vascular tissues of the plum, i. e., that part of the pulp which did not go through the cheese cloth.

†Growth and cellulose hydrolysis are indicated by +, the relative intensities of growth and degrees of hydrolysis being indicated by one or more + marks. Absence of growth and absence of hydrolysis are indicated by —.

cellulose, and, strange to say, *Sclerotinia* is just as active in this respect as *Penicillium*. In many cases the growth was as good on the plum cellulose as on the filter-paper cellulose, yet the hydrolytic action of the fungi was very much weaker on the former medium. No cellulose hydrolysis occurred where peach juice or some soluble carbohydrate, such as glucose, was added. It seemed probable at first that a very small amount of glucose, or peach juice, or sodium citrate would give the fungus a vigorous start and thus accelerate its cyto-hydrolytic activity, but the quantities of these substances employed was sufficient to exert a protective influence, there being a vigorous growth but no apparent cellulose hydrolysis.

The fact that these fungi do not dissolve cellulose, derived either from the host or from paper, when other organic nutrients are supplied, verifies the writer's observation that *Sclerotinia cinerea* does not disintegrate the cell walls of the host tissues. Furthermore, the fact that the fungus dissolves paper cellulose very readily when it is the only carbohydrate supplied, leads one to conclude that the action of the fungus on paper cellulose in a nutrient solution low in carbohydrates is not necessarily a good criterion for judging the behavior of the fungus in the host tissue. In the host tissue there may be a form of cellulose different from that of paper, and it is furthermore very evident that there is present in the fruit an abundance of organic material evidently operating in a protective manner. The fungus fails to produce cytolytic enzymes when grown on plum or paper cellulose to which peach juice or even a very little sugar has been added, but acts vigorously on paper cellulose to which no organic nutrient has been added. It is rather peculiar that both fungi act much more readily on paper cellulose than on cellulose isolated from the fruits which are natural hosts for these organisms.

Sclerotinia cinerea grows very slowly when first transferred to a nutrient medium poor in soluble carbohydrates, very few spores and no aërial mycelium being produced. At the expiration of a week or more one may observe that the fungous mycelium has penetrated the surface layer of the agar, and at the expiration of two to three weeks, in case the fungus is growing on paper-cellulose agar, a clear translucent ring may be observed

in the agar just below the fungous filaments, thus indicating that the cellulose is being hydrolyzed. With increasing age of the fungus, this clear and almost transparent area gradually enlarges downward, although the fungus shows little or no corresponding penetration. At the expiration of three weeks or a month, there is a very distinct, clear, and nearly transparent zone in the medium below the region occupied by the fungous mycelium. Since one could see very distinctly how far the fungous filaments had penetrated into the substrate, it was very evident that the cyto-hydrolytic enzyme had diffused beyond the limits of the mycelium.

The method employed in this investigation for the demonstration of cellulase was the same as that used by Kellerman in his recent work (31) and was utilized to demonstrate the fact that the cyto-hydrolytic enzyme secreted by this fungus penetrates the substrate considerably beyond the limits of the filaments themselves. Tubes containing cellulose agar, in which the fungus had been growing for four weeks, were disinfected externally by washing with a bichloride of mercury solution, and cut off at a point about 12 mm. below the clear portion of the medium. The cotton plug was then flamed and pushed into the tube with a glass rod until the agar was partially shoved out of the cut end of the tube. The clear portion of the agar was then cut into disks about 12 mm. in thickness, which were laid on plates poured with nutrient cellulose agar, great care, of course, being exercised throughout the operation to maintain aseptic conditions. The plates so prepared were then placed in an incubator at 25°C. where they remained for two weeks, at the expiration of which time the cellulose was very distinctly hydrolyzed in a ring about the sterile slices of agar. Microscopic examination confirmed the macroscopic observation that these agar disks were free from any infection.

As might be expected, the activity of the secretion of the enzyme cellulase is influenced by temperature, a fact which is well illustrated by the following experiment: Tubes containing cellulose agar inoculated with the brown-rot fungus were kept at temperatures of 10–12, 16–20, and 24–26°C. respectively, and at the end of twelve days the following results were noted: In the cultures maintained at 10–12°C. no apparent growth or

hydrolysis had taken place; those kept at 16–20°C. showed a good growth but no visible cellulose hydrolysis; and in those maintained at 24–26°C. there was about the same extent of growth as in the preceding series but accompanied by a very evident cellulose hydrolysis, a distinctly clear zone of dissolved cellulose surrounding the region occupied by the fungous mycelium. It is therefore evident that even with approximately the same amount of growth cellulose hydrolysis is much more rapid at the higher temperature.

An effort was made to determine whether or not it is possible to "train up" more active cyto-hydrolytic strains of the *Sclerotinia* and *Penicillium* in question. On the one hand, these fungi were grown for several successive generations on peach-juice agar—a medium in which the organisms show no cytolytic activity. On the other hand, these fungi were cultivated for several successive generations on paper-cellulose agar—a medium which is low in soluble carbohydrates, and one in which the fungi exhibit considerable cytolytic activity. Tubes of paper-cellulose agar were then inoculated with the fungi grown in these two ways and careful observations were made to detect any differences in cyto-hydrolytic activity. No differences developed, however, from which it would appear that the source of cultures of *Sclerotinia* or of *Penicillium* does not materially affect the cellulose-dissolving capacity of these organisms, i. e., each fungus shows the same cellulose-hydrolyzing power whether the organism was cytolytically active during the immediately preceding generations or not.

EFFECT OF THE FUNGUS ON PECTIC SUBSTANCES

The power of organisms to change pectic substances has been considered an important factor in the disintegration and softening of host tissue by certain plant parasites. Before entering into a discussion of the experimental phases of this subject, it will perhaps be well to give some idea of the present status of this question, as well as a very brief resumé of the extensive literature which has accumulated about it.

Fremey (23, 24), in 1840, was the first to report an enzyme acting on pectic substances. This enzyme, which he isolated and called pectase, induced the coagulation of pectin, Fremey attrib-

uting this action of the enzyme to the presence of calcium salts. It is of interest to note that pectase was one of the first plant enzymes to be described. Bertrand and Mallèvre (7, 8) concluded that pectose and pectase are almost universally present in green plants, being especially abundant in the leaves. These authors showed that acidity is an important factor in the inhibition of coagulation of pectic bodies by pectase, and also that either barium, calcium, or strontium is necessary for the action of pectase.

Mangin (35, 36), by microscopic tests, has thrown much light on the nature of the middle lamella and holds that pectose is very pronounced in the cell walls of young tissue. In the older cell walls, on the other hand, this author believes that calcium pectate predominates in the middle lamella, considering that the latter is largely if not entirely composed of this substance and that it frequently collects on the surface of the cell walls adjoining intercellular spaces. Bourquelot (11), and Bourquelot and Hérissey (12) secured a thermo-labile enzyme from barley malt extract which acted upon a solution of pectin (taken from the gentian root), changing the latter in such a way that it was no longer coagulated by pectase. The action of this enzyme, which they called pectinase, was thought by them to be that of converting the pectin into reducing sugar. They also designated as pectinase an enzyme which dissolves the pectic coagulum (the latter has been supposed to be calcium pectate). A good resumé of the status of the chemistry of pectic substances is given by Bigelow and others (10).

A number of investigators have reported upon the action of bacteria on plant cells, including the effects of the organisms on the middle lamella. Winogradsky (55), Behrens (5), and others attributed the changes taking place in the flax plant during retting to the dissolving action which the bacteria exert on the middle lamella. It will be unnecessary to review here any more of the earlier work which has been done along this line, since it has been so thoroughly discussed in the comprehensive publications by Jones (29), and Jones, Harding, and Morse (30) on the soft rot of vegetables. These authors studied the effect of the soft-rot bacillus (*Bacillus carotovorus*) on the host and find that the organism is identical with what has been

designated as *B. oleraceæ* Harrison, and *B. omnivorous* Van Hall, and that it may possibly be identical also with Potter's *B. destructans*. By many tests Jones has shown that this organism secretes an enzyme which causes the disintegration of the host cells by dissolving the middle lamella, which, according to the majority of investigators, is composed of salts of pectic acid. This author has further isolated from pure cultures of the organism an extra-cellular enzyme, which he designated pectinase, that destroys the middle lamella of the cells just as does the growing organism. Jones, therefore, considers this enzyme responsible for the disintegrating action of the bacillus.

In my own work I shall adopt the nomenclature used by Jones (29, 30) and Euler (21), namely, employing pectinase as the term to designate the enzyme inducing coagulation of a pectin solution and also the hydrolysis of calcium pectate, or pectinate.

Methods.—In order to determine the effect of the fungus on the middle lamella I have used two methods, (1) a microscopic study of the effect of the fungus on the host cells, and (2) a study of the effect of the organism on the substances (isolated from the host) which are commonly reported to be constituents of the middle lamella. The first method has been discussed above and may be dismissed here by stating that it yielded no positive evidence that the fungus dissolves the middle lamella. By the second method the problem was studied by isolating pectin from the host and studying the effect of the fungus on it and also on its salts, as, for instance, calcium pectinate.

Pectin was isolated from plums by the following method: Thoroughly ripe fruits were steamed—no water being added, the juice filtered off and treated with Almen's reagent[1] (to precipitate the protein) and with a very dilute solution of oxalic acid (to precipitate the calcium). It was found that under these conditions neither a calcium nor a protein precipitate was thrown down either by Almen's reagent or the oxalic acid, and this procedure, therefore, was deemed unnecessary and was abandoned. The plum juice was carefully filtered through a Buchner filter

[1]Abderhalden, E. Handbuch d. biochem. Arbeitsmethoden **2**: 391–92. 1910. Almen's tannic acid solution is made by treating 4 grams of tannic acid with 8 cc. of a 25 per cent solution of acetic acid, and making up to 190 cc. with 40 or 50 per cent alcohol.

and the filtrate treated with 95 per cent alcohol until a flocculent coagulum of pectin was produced. This pectin was separated by means of a Buchner filter, redissolved in water, reprecipitated with alcohol, again separated by means of a Buchner funnel, and finally dried at a temperature slightly higher than room temperature,—the reprecipitation being for the purpose of purification. It should be noted here that the plums were sufficiently acid to make the addition of hydrochloric acid to the alcohol unnecessary.

Experiments with pectin and pectinase.—From the pectin isolated by the above method a saturated aqueous solution was prepared—some of the mineral nutrient solution[1] minus calcium being added, and the resulting solution rendered sterile by fractional sterilization. Test-tubes of this pectin solution were inoculated with *Sclerotinia cinerea* and *Penicillium expansum* with the result that both organisms produced a rather vigorous growth of mycelium and a few spores. At the expiration of one week the inoculated tubes showed a slight clear area just below the fungous felt due to the coagulation and settling out of the pectin in that part of the solution. The coagulation was at this time somewhat more pronounced in the *Penicillium* cultures than in those of *Sclerotinia,* yet very noticeable in both cases, beginning directly below the fungous felt and progressing toward the bottom of the tube. After two weeks the greater part of the pectin solution was coagulated, the flocculent coagulum, or precipitate, being very different from the precipitate produced in a pectin solution by a calcium salt. It should be emphasized here that every precaution was taken to maintain a calcium-free solution, and when it is considered that the addition of calcium develops a reaction very different from that produced by the enzyme, and, furthermore, that the check gave no coagulation whatever, not even when allowed to stand a month or more, the conclusion would seem to be warranted that calcium is not necessary for the production of a gel by pectinase. Both *Sclerotinia* and *Penicillium*, therefore, produced a coagulum in an aqueous solution of pectin, while no such results were obtained in the controls, thus justifying the conclusion

[1]Nutrient solution employed was the same as mineral nutrient solution A used in preparing cellulose agar, but without the calcium.

that these two fungi are capable of producing pectinase. The cultures were kept at a temperature of 18–20°C.

Experiments with calcium pectinate.—Calcium pectinate was prepared by treating a water solution of pectin with freshly-made limewater (care being exercised to avoid an excess of lime), the product thus obtained being filtered off and thoroughly washed until it was no longer alkaline. The calcium pectinate thus prepared was used in making a pectinate agar in a manner similar to that employed in the preparation of cellulose agar, the same mineral nutrient solution (nutrient A) being used and the whole rendered sterile by fractional sterilization. After the last heating, care was taken to distribute the pectinate, which quickly settles to the bottom of the tubes, uniformly throughout the agar by stirring the medium with a sterile glass rod. These tubes were then inoculated with *Sclerotinia* and with *Penicillium*, the object being to compare the action toward pectic substances of two fungi that have entirely different effects on the host cells, the former producing no softening effects, while the latter causes a very rapid softening and disorganization of the host tissue.

The inoculated tubes of pectinate agar prepared by the above method were kept at a temperature of 22–24°C. Contrary to expectations, there was very little growth when no soluble carbohydrate was supplied, and, furthermore, no dissolving action on the calcium pectinate. On the other hand, when 0.5 per cent glucose was added, both fungi produced a vigorous growth, but neither one gave any indication of pectinate hydrolysis, or dissolution. Here again, as in the cellulose hydrolysis, the two fungi, *Sclerotinia* and *Penicillium*, behave alike. This is not in accordance with the observed behavior of these two organisms toward the host tissue.

ACID RELATIONS OF THE FUNGUS

Some investigators have held that the content of tannin (47) and of malic and other acids of the host determines whether or not the fungus can grow in the tissues and rot the fruit. In accordance with this view a fungus may not so readily attack green as ripe fruit, the former being supposed to exhibit a higher

content of these restraining agents. The question of the acid relation of the host tissue is one of fundamental significance and one that is worthy of considerable investigation; it is important to know to what extent acidity may be a limiting factor in parasitism.

A case in which a certain acid content is favorable for the fungus is developed by Falck (22). He finds the acidity of the substrate to be a conditioning factor for the growth of several species of *Merulius*. In this connection the author observes that *Coniophora*, in particular, acts to pave the way for *Merulius* in that the former organism renders the nutrient substrate decidedly acid, and thereby provides favorable conditions for the germination of the spores and the subsequent growth of mycelium and fruit bodies of *Merulius*. In connection with the investigation of the plum disease here discussed it would be well to know if the acidity of the fruit changes during the progress of its growth, and if so in what direction. It is also essential to know whether or not a change in the acidity of the host can account for the fact that ripe fruit is more susceptible to the disease than green fruit. Some experiments were planned, therefore, to determine to what extent the acidity of the host influences the attack of the parasite, and also to investigate what effects, if any, the fungus has with respect to the acid content of the host.

In order to determine the changes in acidity which take place during the growth of the fruit (plums), several analyses for acidity were made at intervals during the summer. The plums for all of the analyses were taken from the same tree, a known weight of pulp being ground up in a mortar and squeezed through muslin. The acidity was reckoned in the number of cc. of N/10 NaOH required to neutralize one gram of plum pulp. The results were as follows:

June 28, 1 gram plum pulp required 0.66 cc. N/10 NaOH for neutralization,

Aug. 2, 1 gram plum pulp required 2.12 cc. N/10 NaOH for neutralization,

Aug. 19, 1 gram plum pulp required 2.46 cc. N/10 NaOH for neutralization,

the fruit being market ripe on August 19. In these tests my results agree with those obtained by Bigelow and Gore (10) for peaches, and with those of Thompson and Whittier (49) for some other fruits. The last mentioned investigators, however, found that the acidity of peaches decreases toward maturity. I have been unable to secure data covering the acidity of plums throughout the season.

The above results show that the acid content of plums increases rather than diminishes toward the maturity of the fruit. The results of the experiments and field observations show that mature and ripe fruit is much more susceptible than the green and immature fruit. The above facts, showing that as the fruit approaches maturity the acidity increases while the susceptibility to the disease also increases, indicate that there is no close relationship between the low acid content of the host and susceptibility to the brown-rot fungus, and that we must look to other factors to explain infection as observed in the field. As pointed out, my experiments indicate that penetration is a

TABLE III

RELATION OF THE GROWTH OF SCLEROTINIA CINEREA TO THE REACTION OF THE MEDIUM

Medium	Acidity	Growth after 8 days	Growth after 16 days	Spore production
Cherry juice	+2.3*	–†	+†	+
Cherry juice	+1.5	++	++	++
Cherry juice	+1.0	+++	++	++
Cherry juice	+0.15	–	+	+
Cherry juice	–0.15	0	++	0
Cherry juice	–0.30	0	++	0

*Acidity is given in cc. of N/10 NaOH necessary to neutralize 1 cc. of the juice.

†The + sign indicates a fairly good mycelial growth, or spore formation, and the – sign indicates that the growth was just perceptible; 0 indicates no growth, or no spore formation.

very important factor. It is possible that a study of the tannin content[1] might yield some relation of interest.

A preliminary experiment was planned to determine the acidity at which the optimum growth and spore production of the fungus occurs. For this purpose the juice from ripe sour cherries was used. The juice was squeezed out of the cherries (no water being added) and a portion titrated to determine the acidity. Then 50 cc. of this liquid were put into each of a number of Erlenmeyer flasks of 125 cc. capacity; some of the flasks were left untreated, while others received various quantities of N/10 NaOH to bring each to the desired acidity or alkalinity. The flasks were then sterilized and inoculated. The results are given in table III.

It is clear, therefore, that although the fungus eventually grows on a medium as acid as the natural juice of sour cherries, it grows more luxuriantly on a somewhat less acid medium. It is a rather significant fact that on the media near the neutral line the fungus at first shows no perceptible growth, but at the expiration of two weeks has produced nearly as much mycelial growth as on the acid medium. It is also of interest to note that we find spore formation abundant on the very acid media but entirely lacking on the alkaline media. This experiment indicates that the fungus can adjust itself to a slight degree of alkalinity.

OXALIC ACID PRODUCTION BY THE FUNGUS

The first important reference to oxalic acid production by fungi is in the publication by de Bary reviewed in a preceding section. He reports that the older hyphæ of the fungus were encrusted with crystals of oxalic acid, and he attributed some of the poisonous action of the parasite to the production of this substance; in fact, he mentions oxalic acid fermentation. Since the appearance of de Bary's paper a limited number of investi-

[1] Cook and Bassett and their associates (17) believe that there are enzymes in the host plant which may act upon cell constituents and play the rôle of alexins. They are of the opinion that tannin, as such, is not abundant in fruits, but that it may be formed by the action of oxidizing enzymes upon certain phenols. Injuries produced by parasitic fungi may accelerate the activity of the host in the production of tannin, the latter perhaps being toxic to the growth of parasitic fungi.

gators have reported the presence of oxalic acid resulting from the growth of both fungi and bacteria, but unfortunately much of this work is of little value, because methods of analysis are not given. The detection of this acid by some methods is very unsatisfactory.

A few years after de Bary's work, Wehmer (54) published an extensive series of articles on this subject. He studied a number of fungi (mostly saprophytic) with reference to oxalic acid excretion, and of these he found *Aspergillus* to be the most active and *Penicillium* next, and, therefore, he confined his studies to these two fungi. Some of the factors concerned in the production of oxalic acid or its salts, according to Wehmer, may be summed up here: (1) A large yield of oxalic acid is not produced in the presence of free organic or inorganic acids, not being found in the medium when free acids exceeded 0.2–0.3 per cent, while, on the other hand, it can be formed in the presence of as much as 2–3 per cent of the salts of these acids. (2) The sources of nitrogen are very important, for the amount of the oxalic acid produced varies according to the kind and quantity of nitrogenous compounds supplied. (3) Abundant oxalic acid formation is favored by the addition of some basic phosphate, or at least some compound with which the acid can combine to form a soluble salt. (4) The effect of light or darkness on oxalic acid formation is inappreciable. (5) Temperature is an influencing factor in oxalate production, for the latter is inhibited by a high temperature, the temperature for a maximum oxalate production being, in fact, very near the minimum for the growth of the organism.

Wehmer's analytical method consisted in precipitating out the oxalic acid, or its soluble oxalate, as the calcium salt, which was filtered off, dried to a constant weight, and weighed. Although this method is perhaps as well suited for this purpose as any other reported, it is open to criticism. A detailed discussion, however, will not be given here.

Wehmer holds that oxalic acid is a type of excretion, and that it is in some way connected with respiration, that is, with CO_2 elimination. He considers that the variability in the amount of oxalic acid produced is due to its use in the metabolism of the fungus. Emmerling (20), in his contribution to this subject,

emphasizes the influence of such nitrogenous substances as proteins, amino acids, and amides in the nutrient. He finds that *Aspergillus niger* when grown in non-amino acids, for example, tartaric, lactic, etc., produces no oxalic acid, whereas an abundant oxalic acid production results on such substances as peptone or aspartic acid.

Smith (46) and Peltier (41) both conducted experiments to determine whether or not oxalic acid is present in media in which *Botrytis* has been growing. Peltier reported negative results, but Smith found oxalic acid and thinks that the poisoning effect of the fungus is perhaps due to the presence of this acid. Unfortunately, neither of these authors gives his methods of analysis, and, with the exception of one incident in Smith's publication, the quantity of oxalic acid found is not reported. Peltier and others have been able to produce an injury with oxalic acid similar to that produced by certain parasitic fungi, such as *Botrytis*, yet this is not conclusive evidence that oxalic acid is the toxic substance secreted by the organism.

The articles mentioned above constitute the chief publications that have to deal with the production of oxalic acid by fungi. The publications on the production of oxalic acid by bacteria and other plants will not be reviewed here. Whether oxalic acid production is a phenomenon peculiar to certain genera or to certain species of the fungi, whether it is purely the result of external conditions, or whether it results primarily from certain constituents of the medium, has not been clearly demonstrated. A series of experiments was planned in the hope of throwing some light on its production in the fungus here studied.

The method of analysis employed was a modification of Wehmer's method of precipitating the oxalate with calcium chloride and determining the amount of oxalate thus precipitated. This method, however, is not well adapted to the purpose at hand, especially when quantitative methods are used, and fruit juice is employed for the medium on which to grow the fungus. An attempt is being made to develop a method that will be better suited to our purpose.

Culture media were prepared from peaches and plums by filtering the juices of these fruits through a Hill pressure filter under sterile conditions. The product thus obtained was

placed in flasks and incubated for a week and found to be sterile, after which the flasks were inoculated with *Sclerotinia cinerea*. At the expiration of thirty-seven days these cultures were analyzed and were found to contain the following amounts of oxalic acid per 50 cc. of the respective juices:

Plum juice. . . . . 0.0019 grams of oxalic acid,
Peach juice. . . . . 0.0077 grams of oxalic acid,
Peach juice. . . . . 0.0094 grams of oxalic acid,
Control. . . . . No trace of oxalic acid.

Plum and peach juices that had been sterilized by heat, thereby precipitating some of the contained proteinaceous material, were also used as culture media, and here, too, every culture containing the fungus gave a positive test for oxalic acid.

For investigating the production of oxalic acid by the fungus in the unaltered fruit, lots of 500 grams each of peaches were disinfected with bichloride of mercury solution, inoculated respectively with *Sclerotinia*, *Penicillium*, and *Aspergillus niger*, and kept under sterile conditions until the fruits were decayed, or, in the case of the *Penicillium* and *Aspergillus*, until partially decayed. The decayed fruits were then digested with hydrochloric acid and analyzed for their oxalic acid content with the following results:

Peach inoculated with *Penicillium* . . No trace of oxalic acid,
Peach inoculated with *Aspergillus*. . . No trace of oxalic acid,
Peach inoculated with *Sclerotinia cinerea*. . . . . 0.0087 grams of oxalic acid,
Peach control. . . . . No trace of oxalic acid.

The results of these experiments with oxalic acid show that *Sclerotinia cinerea* when grown either on fruit juices or on peaches produces more or less oxalic acid as a result of its metabolism. It is also significant that the other two fungi employed, namely, *Aspergillus* and *Penicillium*, which are not natural parasites on the plum or the peach, produced no oxalic acid under the conditions in which the experiments were carried out.

SUMMARY

1. The brown-rot organism will infect fruits which are immature, even penetrating those which are not more than half-grown or those in which the pits are still soft, provided the

skin is punctured. Infection of green fruits is also effected when a portion of the mycelial felt of the fungus is laid on the surface of the plum. On the other hand, ripe or nearly mature fruits may be readily inoculated by sowing a spore suspension on the unpunctured surface.

2. The fungus does not show any particular affinity for the middle lamella, but penetrates and permeates with equal avidity any part of the host tissue.

3. A study of the effect of the organism on the host gives no positive evidence that a toxic substance is abundantly secreted in advance of penetration.

4. The fungus shows very slight cytolytic action with respect to cellulose isolated from the plum, while, on the other hand, the organism readily hydrolyzes cellulose from filter paper when this is the only carbohydrate supplied. No general cytolytic action of the organism on the cell wall of the host is perceptible.

5. An aqueous solution of pectin isolated from plums was coagulated by *Sclerotinia*, thus indicating the secretion of the enzyme pectinase. In respect to its action on pectic substances, *Sclerotinia cinerea* behaves in a manner similar to that of *Penicillium expansum*, yet these two organisms produce very different effects on the host, the former producing a firm rot and the latter a soft one. Neither organism will dissolve calcium pectinate.

6. The experiments on the acid relations of the fungus indicate that the changing acidity of the host as the fruit reaches maturity does not explain the fact that ripe fruit is more susceptible to the disease than green fruit.

7. The brown-rot fungus produces oxalic acid when grown either on a fruit juice medium or on peaches.

The writer takes pleasure in acknowledging his indebtedness to Professor B. M. Duggar for his advice and helpful criticism in this investigation. Part of this work was done during the summer of 1913 in the Laboratory of Plant Pathology of the University of Wisconsin, and the writer wishes to express his gratitude to Professor L. R. Jones for the courtesy extended to him while at Madison.

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THE THELEPHORACEÆ OF NORTH AMERICA. II[1]

CRATERELLUS

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CRATERELLUS

Craterellus Pers. Myc. Eur. **2**:4. 1825.—Fries, Epicr. 531. 1838; Hym. Eur. 630. 1874.—Saccardo, Syll. Fung. **6**:514. 1888.—Hennings, in Engl. & Prantl, Nat. Pflanzenfam. (1. 1**): 127. 1898.

The type species of the genus is *Craterellus cornucopioides* L. ex Pers.

Fructifications fleshy or membranaceous, pileate, often tubiform, infundibuliform, or flabelliform, sometimes clavate; hymenium waxy-membranous, distinct, continuous, adnate to the hymenophore, even or rugose; basidia simple; spores usually white.

Craterellus is closely related by its fleshy *C. Cantharellus*, *C. odoratus*, *C. lutescens*, etc., with the genus *Cantharellus*. These species resemble so closely in coloration and habit species of the latter genus that careful examination of the hymenium should be made for generic determination. *Craterellus* has its hymenium even or slightly rugose. In exceptional connecting species, such as *C. clavatus*, it is somewhat lamelliform for a part of the distance from margin of the pileus to the stem. The clavate *C. pistillaris* and *C. unicolor* connect *Craterellus* closely with *Clavaria*.

Craterellus cornucopioides, *C. ochrosporus*, *C. clavatus*, *C. Cantharellus*, and *C. odoratus* are edible species, which are often abundant locally.

[1] Issued September 30, 1914.

NOTE.—Explanation in regard to the citation of specimens studied is given in Part I, Ann. Mo. Bot. Gard. 1: 202, footnote. The technical color terms used in this work are those of Ridgway, Color Standards and Nomenclature. Washington, D. C., 1912.

KEY TO THE SPECIES

Hymenium somewhat radiately lamelliform—at least near the margin; stem solid 1
Hymenium plane, rugose-wrinkled, or ribbed and rugose-wrinkled 2

1. Fructification large, 4–10 cm. high; stem about 1 cm. thick; spores 10–13 x 4–4½ μ ..... *1. C. clavatus*
1. Fructification small, 1–1½ cm. high; stem 1 mm. thick; pileus umbilicate; spores 9 x 7 μ ..... *11. C. delitescens*
2. Fructification with pileus infundibuliform and pallid rose; hymenium and stem white. In N. Carolina in moss near *Kalmia* bushes ..... *4. C. roseus*
2. Fructification entirely egg-yellow, about 3–9 cm. high, 2½–9 cm. broad ... 3
2. Fructification neither entirely egg-yellow nor with pileus pallid rose and hymenium and stem white 4
3. Pileus convex, then depressed or infundibuliform; stem solid ... *2. C. Cantharellus*
3. Pileus convex, then depressed or cyathiform; stem hollow or cavernous; fructification sometimes branched ..... *3. C. odoratus*
4. Pileus tubiform with cavity extending nearly or quite to the base of the stem 5
4. Pileus not tubiform, but instead infundibuliform, depressed, truncate, convex, or flabelliform 6
5. Pileus and stem smoky brown to blackish; hymenium cinereous drab; spores 12–16 x 6–10 μ ..... *5. C. cornucopioides*
5. Pileus drying avellaneous to snuff-brown; stem black with chamois-colored pubescence at its base; hymenium chamois-colored or colored like the pileus; spores 12–15 x 7–8 μ ..... *6. C. ochrosporus*
5. Pileus somewhat tubiform; hymenium dark cinereous; spores 6–7½ x 4½–5 μ *7. C. dubius*
5. Pileus somewhat tubiform or umbilicate, yellowish brown to fuscous; hymenium and stem yellow; spores 10–12 x 6–8 μ ..... *8. C. lutescens*
6. Pileus infundibuliform, 2–3 cm. broad; hymenium pallid cinereous; spores 10–12 x 6–7 μ ..... *9. C. sinuosus*
6. Pileus deeply cup-shaped, 4–8 mm. broad; hymenium cream-buff; spores 8 x 6 μ ..... *10. C. calyculus*
6. Pileus convex, then umbilicate, 5 mm. broad; hymenium sometimes obscurely lamelliform, chamois-colored; stem chamois-colored; spores 9 x 7 μ ..... *11. C. delitescens*
6. Pileus merely depressed, truncate, convex, or clavate 7
6. Pileus flabelliform 8
7. Fructification small, 1–3 cm. high, 4–9 mm. broad, narrowly obconic, white; spores 3–4 μ in diameter ..... *12. C. taxophilus*
7. Fructification 2–5 cm. high, from obconic often becoming abruptly enlarged and somewhat cerebriform at the upper end but with the stem remaining comparatively slender ..... *13. C. unicolor*
7. Fructification large, 6–15 cm. high, clavate or obconic and truncate, tapering downward; stem often bulbous at the base. Fructification dries sorghum-brown to fuscous ..... *14. C. pistillaris*
8. Pileus ligulate at first, then spreading laterally and becoming somewhat palmately cleft into a few branches, fawn-color shading into bone-brown. Known from Ohio ..... *15. C. palmatus*

8. Pileus somewhat triangular, drying a dirty pinkish buff; hymenium drying Isabella-color to clay-color. Known only from Florida. . .*16. C. dilatus*
8. Fructification entirely white; pileus reniform, dimidiate, attached laterally to a slender erect stem. Known only from Washington
17. C. Humphreyi

1. **Craterellus clavatus** Pers. ex Fries, Epicr. 533. 1836–1838. Plate 15. fig. 6.

Merulius clavatus Pers. Obs. Myc. **1**: 21. 1896.—*Cantharellus clavatus* Fries, Syst. Myc. **1**: 322. 1821.—*Nevrophyllum clavatum* Fries ex Patouillard, Tab. Anal. Fung. **1**: 193. *f. 434*. 1883–1886.—*Cantharellus brevipes* Peck, Rep. N. Y. State Mus. **33**: 21. *pl. 1. f. 18–20*. 1879.

Illustrations: Schæffer, Icon. Fung. *pl. 164, 276*.—Krombholz, Abbild. und Beschr. *pl. 45. f. 13–17*.—Fries, Sverig. Ätl. Svamp. *pl. 91*.—Richon et Roze, Atlas Champ. *pl. 50. f. 10–14*.—Bresadola, Funghi Manger. *pl. 82*.—Peck, Rep. N. Y. State Mus. **33**: *pl. 1. f. 18–20*.—Harper, Mycologia **5**: *pl. 93, 94*.

Fructifications solitary or cespitose, fleshy, flesh whitish; pileus narrowly obconic, turbinate, truncate or depressed, glabrous, ochraceous buff, attenuated into the stem, the margin thin and erect; stem short, solid, tomentose at the base; hymenium lamelliform near the margin, rugose-wrinkled elsewhere, becoming pruinose with the spores, light vinaceous drab, drying drab; spores pale ochraceous in the mass, 10–13 x 4–4½ μ.

Fructifications 4–10 cm. high; pileus 3–8 cm. broad; stem 1–2 cm. long, 8–15 mm. thick.

On the ground in coniferous woods. Maine to Connecticut and west to Minnesota, and in Montana. July to September.

This species is intermediate between *Craterellus* and *Cantharellus*. The marginal portion of the hymenium is like that of a *Cantharellus*, and the remainder of the hymenium, like that of a *Craterellus*. There is good authority for including this species in *Cantharellus* and there is the authority of Fries and herbarium usage for classing it in *Craterellus*. *C. clavatus* is edible but too rare, at least in the east, to be common in herbaria.

Specimens examined:

Exsiccati: De Thuemen, Myc. Univ., 1807.
Austria: *G. Bresadola*.
Maine: *Sprague* (in Curtis Herb., 5786).

New Hampshire: Shelburne, *W. G. Farlow* (in Mo. Bot. Gard. Herb., 4868).
Vermont: Lake Dunmore, *E. A. Burt.*
Connecticut: Rainbow, *C. C. Hanmer, 1454* (in Hanmer Herb.).
New York: Ballston, *C. H. Peck,* the type of *Cantharellus brevipes* (in Coll. N. Y. State).

2. C. Cantharellus Schw. ex Fries, Epicr. 534. 1836–1838. Plate 15. fig. 7.

Thelephora Cantharella Schw. Schrift. d. Naturforsch. Gesell., Leipzig, **1**: 105. 1822.—*Craterellus lateritius* Berk. Grevillea **1**: 147. 1873.

Illustrations: Peck, Rep. N. Y. State Mus. **49**: *pl. 44. f. 1–5;* Mem. N. Y. State Mus. **3**[4]: *pl. 56. f. 17–21.*—Hard, Mushrooms *f. 378.*—Marshall, Mushroom Book 73. *f.*

Type: in Herb. Schweinitz.

Fructifications single or cespitose, fleshy, firm, egg-yellow; pileus convex, becoming depressed or infundibuliform, glabrous, yellow, the margin often lobed or irregular; stem solid, cylindric or tapering downward, glabrous, yellow; hymenium nearly even or rugose wrinkled, yellow, or with a reddish salmon tinge and drying ochre-red; spores 7–10 x $3\frac{1}{2}$–$5\frac{1}{2}$ μ.

Fructifications 4–9 cm. high; pileus $2\frac{1}{2}$–8 cm. broad; stem $2\frac{1}{2}$–5 cm. long, 5–10 mm. thick.

On the ground in open woods. Massachusetts to Alabama and westward to Ohio; also in Mexico. June to September. Abundant locally.

This species is so similar to *Cantharellus cibarius* in habit, coloration, size and form—differing from the latter only in the more even hymenium, that figures of *C. cibarius* will serve very well for *Craterellus Cantharellus,* if allowance is made for the different hymenium. The firm and solid stem of *C. Cantharellus* distinguishes this species from *C. odoratus* easily. The latter species sometimes has its pileus greatly branched. My illustration of this species is photographed from the dried herbarium specimen of the cotype of *C. lateritius* Berk. In this specimen the lobes of the pileus were pressed together above before drying. The hymenium of this specimen is now ochre-red and agrees in color with that of the authentic specimen of *C. Cantharellus* in Curtis Herb.; both these specimens have been poisoned. I

found the spores of the type in Herb. Schw. 8–9 x $3\frac{1}{2}$–4 μ, or a little slenderer than in northern specimens. Hard states that the spores are yellowish or salmon colored in the mass when collected. This species is edible.

Specimens examined:

Exsiccati: Ell. & Ev., N. Am. Fungi, 1921.

Massachusetts: *Sprague* (in Curtis Herb.); Milton, *H. Webster.*

Connecticut: East Hartford, *C. C. Hanmer, 2391, 2468* (both in Hanmer Herb.).

Pennsylvania: West Chester, *B. M. Everhart,* Ell. & Ev., N. Am. Fungi, 1921.

West Virginia: Eglon, *C. G. Lloyd, 02292.*

North Carolina: *Schweinitz,* type (in Herb. Schweinitz); Blowing Rock, *G. F. Atkinson, 4313.*

South Carolina: Clemson College, *P. H. Rolfs, 1830.*

Alabama: *Peters* (in Curtis Herb., 4539, and in Kew Herb.), the cotype and type respectively of *C. lateritius;* Auburn, *F. S. Earle* (in Mo. Bot. Gard. Herb., 4928).

Ohio: *A. P. Morgan* (in Lloyd Herb.).

Kentucky: *C. G. Lloyd* (in Lloyd Herb.).

Mexico (?): *Botteri, 27* (in Curtis Herb.). If the stem is hollow this specimen is *C. odoratus.*

3. C. odoratus Schw. ex Fries, Epicr. 532. 1836–1838.

Plates 15, 16. figs. 8–10.

Merulius odoratus Schw. Schrift. d. Naturforsch. Gesell., Leipzig, **1**: 91. 1822.—*Cantharellus odoratus* Fries, Elenchus Fung. **1**: 51. 1828.—*C. confluens* Berk. & Curtis, Jour. Linn. Soc. Bot. **9**: 423. 1867.

Type: in Herb. Schweinitz.

Fructifications gregarious, sometimes cespitose, simple or branched, egg-yellow; pileus thin, convex, then depressed and somewhat cyathiform, sometimes pervious, yellow, the margin deflexed, often lobed or irregular; stem cylindric or somewhat tapering towards the base, concolorous with the pileus, hollow or cavernous; hymenium even or somewhat rugose-wrinkled, ochraceous orange or with a reddish tinge approaching Sanford's brown; spores even, 7–9 x 4–5 μ.

Fructifications 3–7 cm. high; pileus 2–9 cm. broad; stem 2–4 cm. long, 3–8 mm. thick.

In moist places in woods. North Carolina and Georgia to Ohio and Missouri. June to October.

Specimens of this species have sometimes been confused in recent years with the better known *C. Cantharellus*, which ranges farther north. The color and general habit of these species is the same; both have the egg-yellow color and the characteristic fragrance of *Cantharellus cibarius* when moistened after drying, and all three are edible. *Craterellus odoratus* is more membranaceous than *C. Cantharellus* and it differs from both this species and *Cantharellus cibarius* in having a hollow or cavernous stem whose pliant walls may be pinched together, like those of a rubber tube, before the specimens are dried. Highly branched forms may occur as shown in pl. 16 fig. 10a; this character was unduly emphasized in the original description. The ample collections in the Glatfelter Herbarium seem to show that *Craterellus odoratus* is the most frequent *Craterellus* in the vicinity of St. Louis. Dr. Glatfelter notes on his collection that he has eaten this species and found it quite good. In pl. 15 fig. 8, I give a figure, natural size, from a photograph of the dried herbarium cotype of *C. confluens* B. & C., to show how close the resemblance is to the specimens of *C. odoratus*, collected at St. Louis and figured in the following plate. The type of *C. confluens* has the hymenium rugose-wrinkled, as is often the case in specimens of *C. odoratus;* its habit, dimensions, structure, coloration, and spores are quite those of *C. odoratus*.

Specimens examined:

North Carolina: Salem, *Schweinitz*, type (in Herb. Schweinitz).
South Carolina: Society Hill, *Ravenel, 192* (in Curtis Herb.).
Georgia: Station cited by Schweinitz.
Alabama: Auburn, *L. M. Underwood.*
Ohio: Oxford, *L. O. Overholts, 1721* (in Overholts Herb.).
Missouri: near St. Louis, *N. M. Glatfelter, 348* (in Mo. Bot. Gard. Herb., 42590), and *J. B. S. Norton* (in Mo. Bot. Gard. Herb., 4926).
Mexico: near Orizaba, *Botteri, 6* (type and cotype in Kew Herb. and Curtis Herb., respectively, of *C. confluens*).

4. **C. roseus** Schw. ex Fries, Epicr. 533. 1836–1838.

Merulius roseus Schw. Schrift. d. Naturforsch. Gesell., Leip-

zig, 1: 91. 1822.—*Cantharellus roseus* Fries, Elenchus Fung. 53. 1828.

Fructifications solitary, somewhat fleshy; pileus infundibuliform, somewhat strigose, pallid rose, the margin lobed and inflexed; stem apparently stuffed, attenuated downward, white; hymenium somewhat rugose, white.

In mosses, especially in proximity to *Kalmia*. North Carolina.

Specimens of this species have the habit of *Cantharellus cibarius* but are thinner. Fries received a specimen of *Craterellus roseus* from Schweinitz and expressed the opinion in 'Elenchus' that the species is good. I have seen no specimens of *C. roseus* and base the above on the original description and the comments by Schweinitz and Fries.

5. C. cornucopioides L. ex Pers. Myc. Eur. **2**: 5. 1825.

Plate 17. fig. 17.

Peziza cornucopioides L. Sp. Pl. 1181. 1753. [1st ed.]—*Elvella cornucopioides* Scop. Fl. Carn. **2**: 476. 1760.—*Merulius cornucopioides* Pers. Syn. Fung. 491. 1801.—*Cantharellus cornucopioides* Fries, Syst. Myc. **1**: 321. 1821.

Illustrations: Vaillant, Botan. Paris. *pl. 13. f. 2, 3.*—Bolton, Hist. Fung. *pl. 103.*—Flor. Dan. *pl. 384, 1260.*—Holmskiold, Fung. Dan. **2**. *pl. 5.*—Sowerby, Brit. Fung. *pl. 74.*—Schæffer, Icon. Fung. *pl. 165.*—Bulliard, Herb. de la France *pl. 150.*—Schnizlein, in Sturm, Deutsch. Flora **3**: fasc. 31. *pl. 5.*—Bresadola, Funghi Manger. 75. *pl. 83.*—Cooke, Brit. Edible Fung. *pl. 11. f. 39.*—Dufour, Atlas Champ. *pl. 70. f. 157.*—Hard, Mushrooms 451. *f. 379.*—Peck, Rep. N. Y. State Mus. **48**: *pl. 24. f. 7–10.*—cf. Saccardo, Syll. Fung. **19**: 478, for other references to illustrations.

Fructifications gregarious or somewhat cespitose; pileus thin, somewhat membranaceous, tubæform, pervious, sometimes granular or minutely squamulose, smoky brown to blackish, usually drying Prout's brown, with the erect, spreading, or decurved margin generally lobed, wavy, or irregular; stem short, hollow, even, blackish brown; hymenium even or rugose-wrinkled, cinereous drab; spores hyaline, even, 12–16 x 6–10 μ.

Fructification 5–8 cm. high; pileus $2\frac{1}{2}$–5 cm. broad; stem 1–3 cm. long, 3–5 mm. thick.

On earth in mixed woods. Canada to South Carolina and westward to Missouri. June to September.

The cornucopia craterellus is well characterized by its cornucopia-shaped or narrowly trumpet-shaped pileus ashy to sooty brown in color, by thin flesh which is somewhat tough and flexile, cinereous drab hymenium which sometimes has a brownish tinge, and black stem. This species is too infrequent to afford more than a few herbarium specimens in the regions where I have collected fungi, but it is reported so plentiful in some states as to be highly regarded as an edible species.

Specimens examined:

Exsiccati: Ravenel, Fung. Car. II. 27; Ellis, N. Am. Fungi, 321; Ell. & Ev., Fung. Col., 1723; Shear, N. Y. Fungi, 49; Rabenhorst-Winter, Fung. Eur., 3640.
Sweden: *L. Romell, 48.*
Canada: *J. Macoun, 72, 73.*
Ontario: Casselman, *J. Macoun, 347.*
Vermont: Grand View Mt., *E. A. Burt.*
Massachusetts: *Sprague, 211* (in Curtis Herb.).
Connecticut: *W. A. Setchell.*
New York: Sand Lake, *C. H. Peck* (in Coll. N. Y. State); Alcove, *C. L. Shear*, Shear's N. Y. Fungi, 49; Ithaca, *H. von Schrenk* (in Mo. Bot. Gard. Herb., 4763, 42584), *W. H. Long, Jr.*, Ell. & Ev., Fung. Col., 1723.
New Jersey: Newfield, *H. Leahy*, Ellis, N. Am. Fungi, 321.
Pennsylvania: locality cited by Schweinitz, Syn. N. Am. Fungi; *W. Herbst* (in Lloyd Herb.).
North Carolina: (in Curtis Herb., 502); locality cited by Schweinitz, Syn. Fung. Car. Sup.
South Carolina: *M. A. Curtis* (in Curtis Herb.).
Ohio: Loveland, *D. L. James*, comm. by U. S. Dept. Agr.
Kentucky: Mammoth Cave, *C. G. Lloyd.*
Missouri: Perryville, *C. H. Demetrio*, Rabenhorst-Winter, Fung. Eur., 3640; Meramec Highlands, *P. Spaulding* (in Mo. Bot. Gard. Herb., 4869).

6. C. ochrosporus Burt, n. sp. Plate 17. fig. 15.
An *C. ocreatus* Pers. Myc. Eur. **2**: 5. *pl. 13. f. 2.* 1825?
Type: in Mo. Bot. Gard. Herb., 42585.
Fructifications gregarious or cespitose; pileus thin, somewhat

membranaceous, tubæform, pervious, minutely floccose-squamulose, drying avellaneous to snuff-brown, the margin erect or decurved; stem short, hollow, black, with chamois-colored pubescence at the base; hymenium even or somewhat rugose, sometimes colored like the pileus but in the type chamois-colored; spores straw-yellow in the mass, even, obtuse, 12–15 x 7–8 μ.

Fructifications 4–7 cm. high; pileus 1–3½ cm. broad, 1–2½ cm. long, 2–4 mm. thick.

On the ground among mosses in woods. New York and Missouri. June to September. Probably abundant in Missouri.

Dr. Glatfelter noted a pleasant minty odor for the specimens. This species closely resembles *C. cornucopioides* in form, but differs from that species in having hymenium, spores, and base of stem yellow. A collection from the same spot from which the type collection came, but made in June two years later, has the hymenium snuff-brown and approaches *C. cornucopioides* in this respect. I am not aware of any data on *C. ocreatus* Pers. except that based on the original description which is cited above. That species has presumably not been collected by European mycologists since the original collection from the environs of Paris a century ago. Our specimens differ from that description in having the stem yellow pubescent at the base and the hymenium somewhat rugose, and they may differ in other characters, e. g., spore colors, etc., not given in the brief description of *C. ocreatus*. Hence I give to our American specimens a distinct name.

Specimens examined:

New York: East Galway, *E. A. Burt.*

Missouri: Meramec Highlands, *N. M. Glatfelter* (in Mo. Bot. Gard. Herb., 42585, type, and 42586–87); Columbia, *B. M. Duggar, 134.*

7. C. dubius Peck, Rep. N. Y. State Mus. **31**: 38. 1879.

Illustrations: Hard, Mushrooms *f. 380.*

Type: in Coll. New York State.

Fructifications solitary or cespitose; pileus thin, infundibuliform or subtubiform, subfibrillose, dark brown or lurid brown, pervious, the margin generally wavy and lobed; stem short, hollow, colored like the hymenium; hymenium dark

cinereous and rugose when moist, the obscure crowded irregular wrinkles abundantly anastomosing, nearly even and paler when dry; spores broadly elliptical or subglobose, 6–7½ x 4½–5 μ.

Fructification 5–7½ cm. high; pileus 2½–5 cm. broad, 4 mm. thick.

On ground in woods. Ontario and New York to Illinois. August to October. Rare.

The specimens of this species have the same coloration as those of *C. cornucopioides* but differ from the latter in having a shorter and more funnel-shaped pileus, and smaller spores. Moffatt reported *C. dubius* as abundant at Glencoe, Illinois.

Specimens examined:

Ontario: Belleville, *J. Macoun, 228* (in Coll. N. Y. State).

New York: Adirondack Mts., *C. H. Peck*, type (in Coll. N. Y. State).

Michigan: Sailor's Encampment, Univ. of Wis. Herb., 46.

8. C. lutescens Pers. ex Fries, Epicr. 532. 1838.

Plate 17. fig. 20.

Merulius lutescens Pers. Syn. Fung. 489. 1801; Albertini & Schweinitz, Consp. Fung. 234. 1805.—*Cantharellus lutescens* Fries, Syst. Myc. **1**: 320. 1821.—*Merulius xanthopus* Pers. Myc. Eur. **2**: 19. *pl. 13. f. 1.* 1825.

Illustrations: Vaillant, Botan. Paris. *pl. 11. f. 9, 10.*—Schæffer, Icon. Fung. *pl. 157.*—Bolton, Hist. Fung. *pl. 105. f. 2.*—Persoon, Myc. Eur. **2**: *pl. 13. f. 1.*—Hennings, in Engl. & Prantl, Nat. Pflanzenfam. (1.1**): 129. *f. 70 H.*—Stevenson, Brit. Hym. **2**: 259.

Fructifications solitary to cespitose; pileus thin, somewhat membranaceous, varying from convex and umbilicate to tubiform or funnel-shaped, often pervious, yellowish brown to fuscous, with margin often lobed or irregular; stem flexuous, cylindric, hollow, yellow, drying ochraceous buff, often hairy at the base; hymenium remotely ribbed, even or rugose-wrinkled, yellow, drying cadmium-yellow to ochraceous buff; spores even, 10–12 x 6–8 μ.

Fructifications 2½–5 cm. high; pileus 1½–3 cm. broad, stem 1½–4 cm. long, 2–4 mm. thick.

On moist ground in woods and swamps. Newfoundland to North Carolina and westward to Michigan. August to October.

This species probably ranks next to *C. cornucopioides* in frequency in the United States. The long and yellow stem readily distinguishes this species from *C. ochrosporus*. Specimens of *Cantharellus infundibuliformis* resemble those of *Craterellus lutescens* in form, size, and color, but those of the former species have true lamellæ.

Specimens examined:

Exsiccati: Ellis, N. Am. Fungi, 1302; De Thuemen, Myc. Univ., 404.

Sweden: Stockholm, *L. Romell, 49;* Femsjö, *L. Romell.*

Newfoundland: Bay of Islands, *A. C. Waghorne, 34* (in Mo. Bot. Gard. Herb.).

New Hampshire: Shelburne, *W. G. Farlow*, Ellis, N. Am. Fungi, 1302, and (in Mo. Bot. Gard. Herb., 4932).

Vermont: Lake Dunmore, *E. A. Burt.*

Massachusetts: Worcester, *G. E. Francis, 100.*

New England: *Sprague, 1689* (in Curtis Herb.).

New York: Sand Lake and Helderberg Mts., *C. H. Peck* (in Coll. N. Y. State); East Galway, *E. A. Burt.*

Pennsylvania: locality cited by Schweinitz, Syn. N. Am. Fungi.

North Carolina: locality cited by Schweinitz, Syn. Fung. Car. Sup.

Michigan: Glen Lake, *C. G. Lloyd, 02462.*

9. C. sinuosus Fries ex Fries, Epicr. 533. 1836–1838.

Cantharellus sinuosus Fries, Syst. Myc. **1**: 319. 1821.

Illustrations: Vaillant, Botan. Paris. *pl. 11. f. 11–23.*—Fries, Icon. Hym. **2**: *pl. 196. f. 2.*—Dangeard, Le Botaniste **4**: 147. *f.*—Gillet, Champ. France Hym. *pl.*

Fructifications cespitose, slightly fleshy; pileus infundibuliform, undulate and floccose, light drab; stem cylindric, stuffed, pallid cinereous; hymenium at length with interwoven wrinkles, pallid cinereous; spores 10–12 x 6–7 μ.

Fructifications 2–3 cm. high; pileus 2–3 cm. broad; stem $1\frac{1}{2}$–2 cm. long, 2–4 mm. thick.

On ground in mixed woods. South Carolina. Rare.

I have seen only dried herbarium specimens of *Craterellus sinuosus*. The spore measurements are those of a specimen from Sweden received from Romell. In this specimen the hymenium has dried somewhat chamois-colored.

Specimens examined:
Exsiccati: Rabenhorst, Fung. Eur., 208 (in Kew Herb.).
Sweden: *L. Romell, 50.*
South Carolina: *Ravenel* (in Curtis Herb., 2982).

C. crispus Fr., sometimes regarded as a variety of *C. sinuosus*, was reported from New England, *Sprague*, by Berkeley & Curtis, Grevillea 1: 147, but the specimen is not satisfactory for study. I do not, therefore, like to include *C. crispus* as one of our species.

10. C. calyculus (B. & C.) Burt, n. comb.

Stereum calyculus Berk. & Curtis, Hooker's Jour. Bot. and Kew Gard. Misc. 1: 238. 1849; Grevillea 1: 161. 1873.

Type: type and cotype in Kew Herb. and Curtis Herb. respectively.

Fructifications somewhat fleshy-membranaceous; pileus thin, deeply cup-shaped, minutely tomentose, drying Saccardo's umber, opaque; stem apparently hollow, cream buff, attenuated below, tomentose at the base; hymenium even or slightly venose, cream buff; spores slightly yellowish under the microscope, even, 8 x 6 μ.

Fructifications 2–3 cm. high; pileus 4–8 mm. broad; stem 1 cm. long, 1–2 mm. thick.

On ground in damp shady woods. North and South Carolina. August and September.

Upon moistening, the type in Kew Herbarium proved too soft and fleshy and the hymenium too waxy for a *Stereum*. The sections have the structure of *Craterellus*. The species is near *C. sinuosus* and may prove to be a small form of this when ample material gives more complete knowledge of the species, but, for the present, I regard *C. calyculus* as a distinct species. I refer to *C. calyculus* a collection made by Professor Atkinson at Blowing Rock, North Carolina, the rough-dried and cespitose specimens of which show a somewhat tubiform pileus and spores 7–8 x 4$\frac{1}{2}$ μ.

Specimens examined:
North Carolina: Blowing Rock, *G. F. Atkinson, 4200.*
South Carolina: Santee River, *Ravenel,* Curtis Herb., 1716 (the type and cotype in Kew Herb. and Curtis Herb. respectively).

11. C. delitescens Burt, n. sp. Plate 17. fig. 18.

Type: in Burt Herb.

Fructifications gregarious, cespitose, somewhat fleshy; pileus thin, convex, then umbilicate, dry, fibrillose, sepia-colored, the margin inrolled; stem equal, solid, glabrous, chamois-colored; hymenium even or sometimes obscurely lamelliform, chamois-colored; spores white, even, broadly ovoid, 9 x 7 μ, borne four to a basidium.

Fructification 10–15 mm. high; pileus 5 mm. broad; stem 10–15 mm. long, 1 mm. thick.

Growing among mosses on very thin soil on rocks by waterfall. Vermont. August.

This species is intermediate between *Cantharellus* and *Craterellus* in its hymenial structure, but, as some of the specimens have the hymenium even and bearing mature spores, I include the species in *Craterellus*. The specimens are much smaller than those of *C. calyculus* and have the pileus becoming merely umbilicate. The little fructifications were well concealed among the mosses; I have found them but once.

Specimens examined:

Vermont: Falls of Lana, Lake Dunmore, *E. A. Burt*, type.

12. C. taxophilus Thom, Bot. Gaz. **37**: 215–19. *f. 1–8*. 1904. Plate 17. fig. 21.

Illustrations: Thom, *ibid. f. 1–8*.

Type: in Cornell Univ. Herb., 15445.

Fructifications single, rarely gregarious, fleshy-membranaceous, entirely white when young, becoming pallid to ochraceous buff with age, drying cinnamon buff; pileus narrowly obconic, slightly viscid, the apex truncate, plane, or depressed and with a thin margin which is erect or expanded; stem solid, equal or tapering downward, flexuous, pruinose, with scattered white hairs at the base; hymenium even, becoming longitudinally rugose-wrinkled with age or upon drying; spores white, even, subglobose, 3–4 μ in diameter, borne four to a basidium.

Fructifications 1–3 cm. high; pileus 4–9 mm. broad; stem $\frac{1}{2}$–2 cm. long, $\frac{1}{2}$–1 mm. thick.

On rotten twigs and leaves under prostrate branches of *Taxus canadensis*. New York. October and November.

This delicate fungus was under observation by Dr. Thom

for a month and is described in detail and beautifully illustrated in connection with his original description in the work cited above. I reproduce merely some simple outline sketches of *C. taxophilus;* this is a very distinct species. The specimens were found in Fall Creek Gorge and nowhere except under prostrate branches of *Taxus*, yet they grew on rotting twigs and leaves of other species as well as on pieces of *Taxus*.

Specimens examined:

New York: Ithaca, *C. Thom,* Cornell Univ. Herb., 15445.

13. C. unicolor Rav. Grevillea **1:** 148. 1873.

Plate 16. fig. 11, 12.

C. corrugis Peck, Bull. Torr. Bot. Club **26:** 69. 1899.

Type: in Ravenel, Fung. Car. II. 26.

Fructifications solitary or cespitose, fleshy, with the flesh white, soft, soon shrinking and leaving the pileus hollow; pileus at first clavate, obtuse, flesh-colored tinted with violet, soon obconic or turbinate, broadly convex or truncate, and often abruptly cerebriform at the upper end, glabrous, ochraceous buff, drying Rood's brown to Natal-brown, the margin obtuse, corrugated by the hymenial wrinkles; stem short, equal or tapering downwards, colored like or a little paler than the pileus; hymenium wrinkled or corrugated, colored like the pileus; spores white, 8–12 x 4–6 μ.

Fructifications 2–5 cm. high; pileus $1\frac{1}{2}$–5 cm. broad; stem 1–$2\frac{1}{2}$ cm. long, 5–8 mm. thick.

On ground in thin woods. Massachusetts, Pennsylvania, and South Carolina. October to January.

This fungus presents strikingly the vagaries in the distribution of fungi. It was originally collected at Black Oak, South Carolina, in 1850, by Ravenel, in sufficient quantity so that he distributed the type collection in his exsiccati. Apparently, this fungus, whenever collected, was referred to other species until 1898, when members of the Boston Mycological Club found it in several localities in Massachusetts and it was adequately described by Peck, as *C. corrugis*, from specimens received from Dr. Francis. I have received no specimens of this species since that season; I searched for it in vain for several years in the adjoining state, Vermont. I have compared the specimens of *C. corrugis*, received from Dr. Francis, with Peck's

type and with the specimens of *C. unicolor* in five different copies of Ravenel's 'Fungi Caroliniani.' *C. corrugis* is certainly the same species as *C. unicolor*. It is very strange that in the interval of nearly half a century from the time of the original collection, *C. unicolor* did not attract attention from an intermediate station.

Specimens examined:

Exsiccati: Ravenel, Fung. Car. II. 26; Ell. & Ev., N. Am. Fungi, 1922a under the name *C. pistillaris*.

Massachusetts: Worcester, *G. E. Francis, 61, 84,* and collection dated Nov. 2, also the type (in Coll. N. Y. State) of *C. corrugis;* Lynn, *H. Webster;* Medford, *Mrs. Page and Mrs. De Long,* ex Herb. Boston Mycological Club, 420; Arlington Heights, *E. A. Burt.*

Pennsylvania: Trexlertown, *W. Herbst,* the *C. clavatus* of his 'Fungal Flora'; West Chester, *B. M. Everhart,* Ell. & Ev., N. Am. Fungi, 1922a.

South Carolina: Black Oak, *Ravenel, 1406* (in Curtis Herb. and in Kew Herb.), and type, Ravenel, Fung, Car. II. 26.

14. **C. pistillaris** Fries, Epicr. 534. 1836–1838.

Plates 16, 17. figs. 13, 14.

Illustrations: Schæffer, Icon. Fung. *pl. 169.*—Harper, Mycologia **5**: 263. *pl. 95.*

Fructifications gregarious, fleshy-spongy, drying sorghum-brown to fuscous; pileus somewhat clavate to turbinate or narrowly obconic, truncate, or somewhat convex, at first yellowish cinnamon, then becoming tinged with fuscous, the edge obtuse; stem solid, paler than the pileus, often bulbous at the base; hymenium corrugated and rugose-wrinkled, colored like the pileus, drying sorghum-brown to fuscous; spores even, 10–12 x 6–8 μ.

Fructifications 6–12 cm. high; pileus 2–3½ cm. broad; stem 3–6 cm. long, 4–12 mm. thick.

On ground in woods under coniferous trees. New Hampshire, Vermont, and Michigan. August to October.

Specimens of this species have so nearly the coloration of *C. unicolor* that those, small and undeveloped, in a collection of *C. pistillaris* cannot readily be distinguished from partially developed specimens of *C. unicolor;* but with age, those of *C.*

unicolor—or at least some of them—have the pileus enlarge abruptly in diameter near the upper end and become abruptly globose-cerebriform on a slender stem, as shown in figs. 11 and 12, while *C. pistillaris* increases in length as well as in diameter, tapers downward more uniformly from the truncate upper end, and may have the stem bulbous at the base.

It is a vexed question with mycologists whether *Craterellus pistillaris* Fr. is *Clavaria pistillaris* L. The specimens which I refer to *Craterellus pistillaris* agree well with specimens of this species in Curtis Herbarium, collected at Upsala, Sweden, in 1853, and communicated by E. P. Fries. Pl. 16 fig. 13 is from a photograph, natural size, of these specimens. Their spores are 9 x 6 μ. The Friesian specimens have the same dark color as our American specimens. Only one of the former shows a bulbous tendency at the base of the stem; in this respect our specimens are more like the illustration of Schæffer, cited above. I believe, therefore, that we have *Craterellus pistillaris* Fr. in our flora. I have collected in mixed frondose woods in Missouri what I refer to *Clavaria pistillaris* as understood by European mycologists. As compared with the former species it is of softer structure, much paler in color, more regularly clavate in form, sometimes splitting at the apex. The illustrations of most European authors agree well in regard to *Clavaria pistillaris*. The colored figures of this species in Batsch, Bulliard, Sturm, Dufour, Flora Danica, Hussey, Krombholz, Quelet, and Sowerby present fructifications of the same habit and bright coloration which we have by Peck, Bull. N. Y. State Mus. **94**: *pl. 93. f. 1–4.* and Mem. N. Y. State Mus. **4**: *pl. 66. f. 15–17.*

Specimens examined:

Sweden: Upsala, *E. P. Fries* (in Curtis Herb.).
Austria: *G. Bresadola.*
New Hampshire: Shelburne, *W. G. Farlow* (in Mo. Bot. Gard. Herb., 4933).
Vermont: Middlebury, *E. A. Burt.*

15. C. palmatus Burt & Overholts, n. sp. Plate 17. fig. 19.

Type: in Mo. Bot. Gard. Herb. and in Overholts Herb.

Fructifications gregarious or perhaps cespitose, fleshy-soft; pileus fawn-color shading into bone-brown towards the stem,

glabrous, flattened and ligulate at first, then spreading out laterally at the apex, and at length somewhat palmately cleft into 2–12 unequal, obtuse, finger-shaped branches; stem curved, solid, equal or somewhat tapering towards the base, bone–brown, sometimes swollen where attached to the substratum; hymenium even or but slightly venose, inferior, colored like the pileus; spores white, even, pyriform, tapering to the base, 6–8 x 3–4 μ.

Fructifications 1–2½ cm. high; pileus 3–15 mm. broad, 1 mm. thick; stem 8–15 mm. long, 1–1½ mm. thick.

On rotten chunks of wood in frondose woods. Ohio. June.

All specimens of the collection except one have the pileus flabelliform; in this exceptional specimen, the pileus is narrowly turbinate, depressed, and with the finger-shaped branches arranged in a circle on the margin, pl. 17 fig. 19b. This species makes for *Craterellus* the same connection between the central-stemmed, cup-shaped type of pileus and the flabelliform type that *Thelephora multipartita* shows in *Thelephora*, and that is common in *Stereum*. The hymenium of the flabelliform specimens of *Craterellus palmatus* is so similar to the upper surface of the pileus in color and consistency that one cannot readily distinguish between these surfaces in the dried specimens. For these reasons, the present species cannot be referred to either *Skepperia* or *Friesula*, and it is of especial interest in showing that *Craterellus* has a natural section of species with flabelliform pileus. The spores of *C. palmatus* are noteworthy.

Specimens examined:

Ohio: Oxford, *L. O. Overholts, 1649*, type (in Mo. Bot. Gard. Herb. and in Overholts Herb.).

16. C. dilatus Burt, n. sp. Plate 17. fig. 16.

Type: in Farlow Herb.

Fructifications single, fleshy; pileus flabelliform, somewhat triangular, glabrous, drying a dirty pinkish buff, the margin somewhat irregularly lobed, crisped, and curving upward; stem solid, equal, flexuous, drying Natal-brown, with white mycelium at the base; hymenium even, drying Isabella-color to clay-color; spores white, even, broadly ovoid, obtuse, 8–10 x 6–7 μ.

Dried fructification 4 cm. long; pileus 15 mm. long, 15 mm. broad, ½ mm. thick; stem 2½ cm. long, hardly 1 mm. thick.

On sandy ground in swamp. Florida. September.

Only a single fructification was collected; the description is based upon this dried specimen. The species is distinguished by its fan-shaped, triangular pileus and the comparatively long and slender stem. Its characters are those of a true *Craterellus* and yet such that we cannot regard it as a flabellate form of any other species.

Specimens examined:

Florida: Sorrento Swamp, *R. Thaxter*, type (in Farlow Herb.).

17. C. Humphreyi Burt, n. sp. Plate 17. fig. 22.

Type: in Burt Herb. and in Humphrey Herb.

Fructifications gregarious, fleshy, moderately tough and flexible, entirely white, usually with the pileus standing out horizontally at the apex of the erect stem; pileus reniform, dimidiate, sometimes clasping behind, convex, becoming plane or somewhat depressed, usually even, dry, minutely pubescent, the margin entire, even or slightly crisped; stem lateral, erect, often bent at right angles just before joining the pileus, cylindric below, equal, solid, pubescent; hymenium nearly even, sometimes radiately venose near the stem, brittle when fresh; spores white, even, subglobose, $3\frac{1}{2}$–$4\frac{1}{2}$ x $3\frac{1}{2}$ μ.

Fructifications 3–7 cm. high; pileus 6 mm. – 2 cm. long, 1–$3\frac{1}{2}$ cm. broad, $\frac{3}{4}$ mm. thick; stem $2\frac{1}{2}$–6 cm. long, 2 mm. thick.

On humus and among mosses in low swampy thicket. Washington. October.

The habit of this curious species is very suggestive of *Hydnum auriscalpium;* many of the specimens have the erect stem bent at right angles near the apex so that the pileus extends out in a horizontal plane. Sometimes the stem branches at its upper end and bears two pilei. The pubescence on the stem is rather coarse and is most abundant towards the base. All parts of the fructification were rather brittle in vegetative condition, and broke when bent too far. It is a connecting species between *Craterellus* and *Arrhenia,* but with the hymenium rather too even for *Arrhenia,* in my opinion.

Specimens examined:

Washington: Hoquiam, *C. J. Humphrey, 1386,* type.

Berkeley & Curtis, Jour. Linn. Soc. Bot. **10**: 328, described three species of *Craterellus* from Cuba, which have been transferred to other genera by Patouillard, Bull. Soc. Myc. France **15**: 193–94. *pl. 9*, as follows: *C. spathularius* to *Skepperia* and *C. marasmioides* and *C. pulverulentus* to *Cymatella*. I have received no collections referable to these genera and defer their consideration to the final part of my monograph in the hope that some specimens may be received in the meantime.

Craterellus canadensis Kl. ex Saccardo, Syll. Fung. **6**: 519. 1888, was published by Berkeley, Ann. Nat. Hist. **3**: 380. 1839, under the name *Cantharellus canadensis* Kl. from a specimen in Hooker Herb. bearing manuscript notes by Klotzsch. The specimen was collected in Canada by Richardson. In connection with the original description, Berkeley noted that the nearest affinities of *C. canadensis* are with *C. clavatus*. In 1856, after studying the specimens in Herb. Schweinitz, Berkeley & Curtis, Jour. Acad. Nat. Sci., Phila. N. S. **3**: 206. 1856, note that *Cantharellus canadensis* Kl. is apparently the same species as *Cantharellus floccosus* Schw. I have seen no specimens of *C. canadensis* and follow Berkeley's final disposition of the species.

(To be continued.)

Explanation of Plate

PLATE 15

All figures of this plate have been reproduced natural size from photographs of dried herbarium specimens.

Fig. 1. *Thelephora cæspitulans*. From authentic specimen in Curtis Herb., collected by Schweinitz in North Carolina.

Fig. 2. *T. lutosa*. From authentic specimen in Curtis Herb., collected by Schweinitz in North Carolina.

Fig. 3. *T. dentosa*. From cotype in Curtis Herb., collected in Cuba by C. Wright.

Fig. 4. *T. perplexa*. From type in Curtis Herb., collected in Cuba by C. Wright, 238. *a* shows a resupinate portion, and *b*, an ascending portion of the specimen.

Fig. 5. *T. cornucopioides*. From specimen collected in Castleton Gardens, Jamaica, by F. S. Earle, 238.

Fig. 6. *Craterellus clavatus*. From specimen collected at Lake Dunmore, Vt.

Fig. 7. *C. Cantharellus*. From the cotype in Curtis Herb., 4539, of *C. lateritius*, collected in Alabama, by Peters.

Fig. 8. *C. odoratus*. From the cotype in Curtis Herb. of *C. confluens*, collected near Orizaba, Mexico, by Botteri, 6.

Fig. 9. *C. odoratus*. From the specimens in Curtis Herb., collected at Society Hill, S. Carolina, by Ravenel, 192.

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1. THELEPHORA CAESPITULANS. — 2. T. LUTOSA. — 3. T. DENTOSA. — 4. T. PERPLEXA — 5. T. CORNUCOPIOIDES. — 6. CRATERELLUS CLAVATUS. — 7. C. CANTHARELLUS. — 8 AND 9. C. ODORATUS.

COCKAYNE, BOSTON

EXPLANATION OF PLATE

PLATE 16

All figures of this plate have been reproduced natural size from photographs of dried herbarium specimens, but in the case of fig. 10 the specimens were moistened.

Fig. 10. *C. odoratus.* From specimens collected near St. Louis, Mo., by N. M. Glatfelter, 348. The rough dried specimens were moistened before being photographed. *a* shows a branched specimen; *b*, a fructification split longitudinally to show extent of depression of the pileus and the hollow stem; *c*, view of hymenium.

Fig. 11. *C. unicolor.* From authentic specimen in Curtis Herb., collected at Black Oak, S. Carolina, by Ravenel, 1406.

Fig. 12. *C. unicolor.* From specimen of *C. corrugis* collected at Medford, Mass., by Mrs. Page and Mrs. DeLong.

Fig. 13. *C. pistillaris.* From specimen in Curtis Herb., collected at Upsala, Sweden, by E. P. Fries.

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10. CRATERELLUS ODORATUS. — 11 AND 12. C. UNICOLOR. — 13. C. PISTILLARIS.

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EXPLANATION OF PLATE

PLATE 17

All figures are natural size. Figures 14–20 are from photographs of dried herbarium specimens, but which were moistened before being photographed in case of specimens used for figs. 15 and 17.

Fig. 14. *C. pistillaris*. From specimen collected under hemlock (*Tsuga*) tree, at Middlebury, Vt.

Fig. 15. *C. ochrosporus*. From type specimens in Mo. Bot. Gard. Herb., collected near St. Louis, Mo., by N. M. Glatfelter, 1253. *a* is split longitudinally to show the depth of depression of the pileus; *b*, side view.

Fig. 16. *C. dilatus*. From type in Farlow Herb., collected at Sorrento Swamp, Florida, by R. Thaxter. *a* shows upper surface of pileus, and *b*, the hymenium.

Fig. 17. *C. cornucopioides*. From specimen collected in Canada, by J. Macoun, 72.

Fig. 18. *C. delitescens*. From type specimens collected at Lake Dunmore, Vt.

Fig. 19. *C. palmatus*. From type specimens in Mo. Bot. Gard. Herb. and Overholts Herb., collected at Oxford, Ohio, by L. O. Overholts, 1649. *a* shows specimens having flabelliform pileus, and *b*, a specimen with turbinate pileus.

Fig. 20. *C. lutescens*. *a* shows hymenium of specimen collected at Shelburne, New Hampshire, by W. G. Farlow, and *b*, upper surface of specimen collected at Lake Dunmore, Vt.

Fig. 21. *C. taxophilus*. From sketches of photographs of type specimens when in vegetative condition, collected at Ithaca, New York, by C. Thom.

Fig. 22. *C. Humphreyi*. From sketches of the type specimens when in vegetative condition, collected at Hoquiam, Wash., by C. J. Humphrey, 1386.

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14. CRATERELLUS PISTILLARIS. — 15. C. OCHROSPORUS. — 16. C. DILATUS. — 17. C. CORNUCOPIOIDES. — 18. C. DELITESCENS. — 19. C. PALMATUS. — 20. C. LUTESCENS. — 21. C. TAXOPHILUS. — 22. C. HUMPHREYI.

COCKAYNE, BOSTON

THE EFFECTS OF SURFACE FILMS ON THE RATE OF TRANSPIRATION: EXPERIMENTS WITH POTTED POTATOES

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In a previous report[1] we have presented data which is believed to justify the conclusion that an application of a surface film of Bordeaux mixture to the leaves of the castor bean or the tomato increases materially the rate of transpiration. The importance of a careful determination of various physiological effects of this spray mixture was suggested primarily by the increased vitality and yield exhibited by potatoes (*Solanum tuberosum*) treated with this fungicide during seasons when fungi and insects were unimportant factors. In our previous experiments the potato was not included, and it seemed most important, as a next step, to ascertain the effects of certain sprays upon the transpiration of this plant.

Experience has demonstrated that the potato may not be used satisfactorily in potometer experiments. Moreover, it was desired to arrange the experiment so that the transpiration quantities obtained might represent an interval of a week or more. On the other hand, it had been found as a result of our previous work with potted tomatoes that a very considerable amount of labor is required when it becomes necessary to add measured quantities of water every day to a series of fifty or more potted plants. Accordingly, for this and for other work proposed, a method was devised whereby we were able to employ a self-watering device based on a principle often used in the laboratory.

[1] Duggar, B. M., and Cooley, J. S. The effect of surface films and dusts on the rate of transpiration. Ann. Mo. Bot. Gard. **1**:1–22. *pl. 1*. 1914.

The apparatus is shown in pl. 18. The rack, or support, is made of a single sheet of galvanized iron 18 cm. wide and 55 cm. long, these dimensions being adequate for a stand 33 cm. high. Besides cutting a hole in the upper part for the insertion of the neck of the bottle, the operation of making a stand will be clear from the plate and involves merely a few slits with the shears, the balance being accomplished by bending. Two or four rivets may be used if additional strength is required. With regard to other features of the apparatus it is well to note that (1) the shoulder of the flower pot rests on the rim of a tin cup somewhat deeper than the pot, the latter containing the immediate supply of water; (2) there is an inverted bottle with a capacity of about 1500 cc. serving as a reservoir of water and aspirator; and (3) the bottle is connected with the cup by glass and rubber tubing.

In setting up an experiment the exposed area of the pot (above the shoulder) and the soil are covered with paraffin or parawax; the cup is filled with water to such height that when the pot is inserted the water will rise to the height of about 2 cm. on the side of the pot, thus insuring adequate absorption; while a notch in the side of the cup makes it possible to introduce the rubber tube connecting with the bottle, this tube being adjusted to reach just below the new level of water in the cup. With a tube of proper diameter, the water level in the cup is kept practically constant so long as the bottle contains water. This apparatus, complete, may be quickly and sufficiently accurately weighed on the Troemner scales. To prevent upsetting, after arranging in the experimental area, it is well to make the stand secure by providing a small hole in the base, through which a bamboo stick may be thrust into the soil. To this stake, also, for further support, the bottle may be fastened by cord or rubber band.

The device above described has saved much time and has enabled us to obtain a soil moisture content practically uniform in all the pots used in the experiment. It possesses the disadvantage of tending to maintain a moisture content which for long-term cultures is too high for the best growth of the potato. A slight modification of the method would seem to be practicable in several aspects of transpiration work.

Three weeks before the experiment began the plants were repotted, new 5-inch pots of good quality being used, and at the time of the installation of the experiment the drainage holes in the pots were carefully corked, so that all transfer of water would be through the porous walls. The potato plants employed were grown in the greenhouse during the early spring, but on April 20, about two weeks before the test was made, they were placed outside, to insure hardiness. When used, the plants were from 25 to 45 cm. high, each plant with from about 15 to 30 leaves. Some plants were blossoming, and tubers were forming.

The experiment embraced 7 series, or lots, of 10 plants each, sprayed with mixtures as follows: (1) strong Bordeaux, (2) control, no spray, (3) weak Bordeaux, (4) lime wash, (5) lime sulfur, (6) strong Bordeaux and lampblack, and (7) lime wash and lampblack. The strong Bordeaux (designated hereafter Bordeaux) contained 12 grams $CuSO_4$ and 14.4 grams CaO per liter of water, being approximately the 5–6–50 formula of agricultural practise. It was made up in the usual way. The weak Bordeaux was one-half the strength of the stronger mixture. The lime wash was a $Ca(OH)_2$ suspension consisting of 60 grams of CaO per liter of water. A commercial preparation of lime sulfur was used, and this was diluted, as usual, to about 1–25. The Bordeaux-lampblack and the lime-wash-lampblack preparations were made by rubbing into small quantities of the Bordeaux and lime wash 5 and 10 grams respectively of lampblack, then diluting to one liter.

The method of selecting the plants for the different lots was precisely that described in the previous report, that is, selecting at one time 7 plants (as many as there were lots) between which there could be little or no choice, and distributing these at random, 1 to each lot until each included 10 plants. All plants (except controls) were sprayed on May 5, but a rain that night, before protection was provided, necessitated respraying the following day. After spraying, the plants were placed on the stands and each connected with its water supply. They were arranged on an exposed lawn, each lot occupying a row, with the plants 4 feet apart. Moreover, several rows of potted potatoes were arranged around the entire area in order that all

plants in the experimental area might have equal exposure. Over the experimental plot a frame was provided, so that the whole area might be protected by tarpaulins in case of rain. Fortunately, however, no rain occurred during the period of the experiment.

After a preliminary exposure of 24 hours, which enabled us to determine that the 70 plants of the experimental area were in good condition, the initial weighings were made. A definite order was established, this being crosswise of the different lots. The same order was observed at the close of the period, and similarly in the second period a consistent scheme was followed, in order that the time interval might be as uniform as possible. After the weighings at the close of the first period, all plants were discarded which showed any signs of weakness or injury arising from the conditions of the experiment. It should be stated, too, that these conditions were taxing. The weather was bright and warm, the pots were severely exposed, and, as already noted, the water content of the pots was necessarily fairly high. With the plants remaining in a condition apparently normal and vigorous from the first period, a second "run" was made, the latter including from 4 to 7 plants in each lot. In selecting plants for this second period, the size factor was again taken into consideration, as far as possible.

TABLE SHOWING WATER LOSS AND GREEN WEIGHT OF THE PLANTS

Lot	Film covering	1st period, May 6–11, 10 plants			2nd period, May 11–15, 7 plants		
		Ave. water loss per plant	Ave. green weight per plant	Water loss per g., green weight	Ave. water loss per plant	Ave. green weight per plant	Water loss per g., green weight
1	Bordeaux, strong	526.6	50.3	10.46	463.3	55.0	8.42
2	Control	413.8	61.0	6.78	433.3	63.1	6.86
3	Bordeaux, weak	642.4	60.9	10.54	574.0	61.7	9.30
4	Lime wash	584.5	70.7	8.27	613.6	76.3	8.04
5	Lime sulfur	443.0	62.8	7.06	450.7	70.0	6.44
6	Bordeaux and lampblack	792.1	66.1	11.97	653.0	75.2	8.69
7	Lime and lampblack	596.6	58.3	10.20	585.6	66.8	8.78

More stress should, however, be laid upon the data from the first period. The green weights of the plants discarded at the close of the first period were taken immediately, while those plants used in the second period could not be weighed until the close of that interval. This small interval of time, however, could cause no material change in the weights. In the accompanying table there are given in grams the average water loss per plant, the average green weight per plant, and the water loss per gram of green matter.

From the data exhibited it is obvious that with potted potatoes, as with castor bean leaves and potted tomatoes in our earlier experiments, there is a marked acceleration of transpiration induced by spraying with Bordeaux mixture, as also with some other films. Of the several films employed, lime sulfur alone yields an average water loss comparable with that of unsprayed plants. Of all lots showing increased transpiration those treated with weak Bordeaux and lime wash were in some respects most satisfactory, inasmuch as the plants used, like those in the control, were, in general, in very good condition throughout the period of the experiment. On the other hand, those treated with the stronger Bordeaux, the Bordeaux and lampblack, and the lime and lampblack gave, towards the close of the periods, evidences of the injurious effects of the increased transpiration (apparently) upon the vitality of the plants. These statements may not seem to be in entire accord with the figures presented, for during the second period of the experiment, for example, the transpiration quantity is relatively greatest in the case of those plants sprayed with weak Bordeaux mixture. Nevertheless, our observations enabled us to predict that certain lots, especially numbers 1 and 6, would give in the second period, particularly, transpiration values less than might be anticipated. The smaller quantities in the lots referred to, as contrasted with the weak Bordeaux, are to be explained, in fact, as a direct result of incipient wilting and slight injury, brought about by the higher transpiration capacity induced under conditions already accentuating transpiration.

It is believed, in the first place, that the experiments here reported confirm our earlier conclusion, namely, that a film of Bordeaux mixture facilitates water loss; but, in the second place,

treatment with a fairly thick lime wash or lime wash and lampblack also increases the transpiration rates, the latter more than the former. Lampblack added to Bordeaux seems also to give a higher rate than the Bordeaux alone. It is to be emphasized, however, that the strength of the lime wash employed is four times as great as the lime in the stronger Bordeaux mixture; likewise, more lampblack is used with the lime wash than with the Bordeaux. It seems to be definitely established that certain specific characters of the film are important, but these results suggest, further, that the additional quality of color is a factor requiring consideration. The fact that injury may result from the accelerated transpiration induced by a heavy film of Bordeaux under the conditions of our experiment does not mean that under normal conditions of growth in the field a benefit may not accrue to certain plants—from factors associated with a high transpiration rate.

Graduate Laboratory, Missouri Botanical Garden.

Explanation of Plate

PLATE 18

View of the apparatus (with tomato plant) by means of which watering was automatically controlled. It has been found convenient to have both stand and cup painted green. For description see text, p. 322.

DUGGAR AND COOLEY — TRANSPIRATION

COCKAYNE BOSTON

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THE THELEPHORACEÆ OF NORTH AMERICA III[1]

CRATERELLUS BOREALIS AND CYPHELLA

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Since the publication of Part II, on *Craterellus*, Dr. Farlow has very kindly called my attention to, and permitted me to study, a specimen of a rare species from Labrador which was not included in my account of our North American species. This species is now described here so as to bring its description and illustration continuous with those of our other species of *Craterellus*.

The following is suggested for insertion in "Key to the Species," on page 328 (Ann. Mo. Bot. Gard. 1: 328. 1914).

6. Pileus membranaceous, infundibuliform, pale buff; hymenium pale buff; spores 5–7 x 4–5½ μ; from Labrador. See page 357 (Ann. Mo. Bot. Gard. 1: 357. 1914)..... *C. borealis*.

Craterellus borealis Burt, n. sp. Plate 19. fig. 1.

Type: in Farlow Herb.

Fructifications solitary, small; pileus infundibuliform, tapering uniformly to the stem, glabrous, drying between cartridge buff and cream-buff, the margin entire; stem nearly equal,

NOTE.—Explanation in regard to the citation of specimens studied is given in Part I, Ann. Mo. Bot. Gard. 1: 202. 1914, footnote. The technical color terms used in this work are those of Ridgway, Color Standards and Nomenclature. Washington, D. C., 1912.

[1] Issued January 30, 1915.

slender, minutely downy, pale mouse-gray; hymenium colored like the pileus, remotely ribbed, with the ribs radiating from the stem, thin, branching; spores colorless, even, 5–7 x 4–5½ μ.

Fructification 2 cm. high; pileus 1 cm. broad, 13 mm. long; stem 7 mm. long, ½ mm. thick, enlarging to 1 mm. where joining the pileus.

In moss. Labrador. August 8, 1908.

The above description is based on the single dried specimen collected by the Bryant Labrador Expedition. The small size, regular obconic form, and very pale color of the membranaceous pileus and the slender stem are characters making *C. borealis* clearly distinct from other species of *Craterellus*.

Specimens examined:

Labrador: Gready Harbor, Gready Island, *Owen Bryant*, type (in Farlow Herb.).

CYPHELLA

Cyphella Fries, Syst. Myc. 2: 201. 1823.

Fructifications somewhat membranaceous, cup-shaped, rarely plane, adnate behind, commonly extended in stem-like form, pendulous; hymenium typically concave or disk-shaped, definitely inferior in the pendulous species, even or at length rugulose; basidia typically four-spored; spores subovate or globose, hyaline, rarely colored.

C. digitalis Fries is the type species of this genus.

The fructifications of all our North American species are comparatively small, ranging in diameter from a fraction of a millimeter for some species to five to fifteen millimeters for those of the largest species. The fructifications are produced on the bark of small rotting twigs on the ground and on dead herbage, and can only be distinguished from small *Peziza* by demonstrating basidia rather than asci in the hymenium. This demonstration is simply made by crushing under a cover glass a portion of a fructification in water containing a little seven per cent solution of potassium hydrate, and then examining the preparation with the compound microscope. The basidia are usually four-spored; in a few species I have as yet been able to detect only two-spored basidia.

Cyphella is closely related to *Solenia* by such species as *C. fasciculata* and *C. mellea*, but is separated from it in such cases

by the absence of a hyphal subiculum over the area on which the fructifications are distributed, and by the less cylindric form of fructifications of *Cyphella*. *Cyphella* is allied to *Merulius* by *C. muscigena* and also to *Craterellus* by this species, specimens of which were described as a *Craterellus*.

A few species of *Cyphella* are common and widely distributed, but most of our North American species are apparently extremely local and are known only from their respective type collections. The lack of specimens available for carrying about to compare with types has been a serious disadvantage in my study of this genus. Basidia and basidiospores have not as yet been found for some species which, although originally referred to *Cyphella*, have to be regarded as even doubtful *Basidiomycetes*. I have supplemented the original descriptions with measurements of dried fructifications and with such data in regard to basidia and spores as the specimens afford. In the case of very scanty types, the few fructifications are too precious for gross comparison to be used for microscopic study. For such species, it seems to me that the descriptions should stand on the original data, without prejudice, until new collections become available. Such imperfectly known and partially described species are grouped together under the heading "Species Imperfectly Known." *Cyphella convoluta* Cke., *C. Cupressi* (Schw.) Fries and *C. subcyanea* Ell. & Ev. are excluded species.

Key to the Species

Fructifications sulphur-colored; hymenium even; spores 4½x2½–3 μ ..... *1. C. sulphurea*
Fructifications sulphur-colored; hymenium minutely pitted; spores 6–8 x 3–4 μ ..... *2. C. læta*
Fructifications white or whitish; on mosses 1
Fructifications white; not on mosses 2
Fructifications neither white nor sulphur-colored 3

1. Fructifications helmet-shaped; hymenium slightly wrinkled; spores 10 x 8 μ ..... *3. C. galeata*
1. Fructifications flattened, irregular in form, sometimes stipitate; spores 3–5 x 2–3 μ ..... *4. C. muscigena*
1. Fructifications seated upon or developing from webby strings of mycelium ..... *5. C. arachnoidea*
2. Fructifications villose, not easily crushed, with a firm base or a short stem; spores 12–18 x 6–6½ μ ..... *6. C. Tiliæ*
2. Fructifications villose, easily crushed, sessile; spores 10–12 x 5–7 μ ..... *7. C. villosa*
2. Fructifications whitish, minutely webby-hairy, easily crushed, sessile; spores 8–13 x 4 μ ..... *8. C. caricina*

2. Fructifications glabrous, with an oblique stem; spores 4½–6 x 3–3½ μ *9. C. capula*
2. Fructifications villose, snow-white, sessile, very minute and delicate; spores 5–6 x 4–4½ μ; from New England.....*10. C. minutissima*
2. Compare with *C. cinereo-fusca, C. Palmarum, C. Peckii, C. perexigua, C. pezizoides* and *C. trachychæta* of "Species Imperfectly Known."

3. Fructifications wholly pale ivory-yellow, downy-pubescent, cup-shaped, sessile; spores 4–7 x 3–4 μ.....*11. C. Langloisii*
3. Fructifications wholly cream-color, not hairy, helmet-shaped, sessile, resupinate-reflexed; hymenium wrinkled; spores 7½ x 4½ μ; on prickle-bearing stems, Jamaica.....*12. C. porrigens*
3. Fructifications mineral-gray, tomentose, cup-shaped, sessile; hymenium fuscous; spores angular, 4½–6 x 4½ μ; on *Juniperus*.....*13. C. cupulæformis*
3. Fructifications wholly gray-pallid, flocculose, sessile; spores 4 x 3 μ.....*14. C. griseo-pallida*
3. Fructifications externally cinereous, farinaceous, flattened, sessile; hymenium convex, brown; spores 8 x 3½ μ; on *Alnus*.....*15. C. subgelatinosa*
3. Fructifications darker colored than the above..... 4

4. Fructifications vinaceous-buff, hairy, sessile, ⅓ mm. broad; spores 10–12 x 6–8 μ; on bark of *Carya*.....*16. C. Ravenelii*
4. Fructifications drying Isabella-color, hairy, sessile, 1–1½ mm. broad; spores 13 x 8 μ; on *Quercus*.....*17. C. texensis*
4. Fructifications Isabella-color, hairy, sessile, ⅓–½ mm. broad; some spores colored, 5–6 x 4–4½ μ; on *Salix*.....*18. C. mellea*
4. Fructifications tawny-olive, tomentose, stipitate; often cespitose; spores 7–9 x 2–2½ μ; usually on *Alnus*.....*19. C. fasciculata*
4. Fructifications fuscous when moist, drying mouse-gray, cespitose and sessile on a common short trunk, glabrous, structure gelatinous *20. C. conglobata*
4. Fructifications sepia or olive-brown, cup-shaped, probably glabrous, sessile or with a very short stem; spores 6–8 x 3½–4 μ; on rotting leaves of *Gladiolus*.....*21. C. fumosa*
4. Compare *C. Bananæ, C. filicicola* and *C. musæcola* in "Species Imperfectly Known."

1. C. sulphurea Batsch ex Fries, Hym. Eur. 665. 1874.

Peziza sulphurea Batsch, Elenchus Fung. Contin. 1: 209. *pl. 27. f. 146.* 1786.—*P. campanula* Nees, System d. Pilze 268. *f. 295.* 1816.—*Cyphella sulphurea* Batsch, in Patouillard, Tab. Anal. Fung. 114. *f. 256.* 1883; Peck, Rep. N. Y. State Mus. **31**: 38. 1879.

Illustrations: Batsch, Elenchus Fung. Contin. *pl. 27. f. 146.* —Nees, System d. Pilze *f. 295.*—Patouillard, Tab. Anal. Fung. *f. 256.*—Oudemans, Ned. Kruidk. Archief III. **2**: *pl. 3. f. 1–5.*

Fructifications scattered or gregarious, membranaceous, broadly campanulate, somewhat irregular, extended into a short stem, even, glabrous, sulphur-yellow, the margin somewhat repand; hymenium even; basidia cylindric, 16 x 4½ μ, 4-spored;

spores colorless, even, broadly ovoid, somewhat flattened on one side, $4\frac{1}{2}$ x $2\frac{1}{2}$–3 μ.

Fructifications about 2–3 mm. high; pileus 1–2 mm. broad; stem 1 mm. long, $\frac{1}{4}$ mm. thick.

On living stems of herbs in damp places. New York. September. Rare.

The minimum dimensions given above for the fructifications are about those of European specimens of this species as figured; the American specimens run rather larger in Peck's collection. Peck noted that some of his specimens were white when collected, but that they dried yellow like the others of the collection. In other respects our American specimens agree closely with the figures and description of European specimens. Oudemans gives the spore dimensions as 10–12 x 4–5 μ, but Patouillard gives them as they are in American specimens.

Specimens examined:

New York: Griffins, Delaware Co., *C. H. Peck* (in Coll. N. Y. State).

2. C. læta Fries, Epicr. 568. 1836–1838.

Illustrations: Patouillard, Tab. Anal. Fung. *f. 362.*

Fructifications membranaceous, obliquely cup-shaped, extended at the vertex into a stem, pendulous, entire, everywhere glabrous and sulphur-colored; stem straight or somewhat flexuous, hymenium minutely pitted; spores colorless, even, 6–8 x 3–4 μ, borne four to a basidium.

Fructifications 3–5 mm. high, 2–4 mm. broad; stem 1–2 mm. long, about $\frac{1}{2}$ mm. thick.

On dead stems of large herbs lying on the ground. New York. August.

Fries described the fructifications as 6–8 mm. broad; the dimensions given above are those of Patouillard's figures and of the specimens collected by Peck. Patouillard notes that the specimens blacken when old; Peck states, "The beautiful sulphur-color is lost in drying." The pitted surface of the hymenium is a noteworthy character of *C. læta* and this and the larger spores of *C. læta* distinguish it from *C. sulphurea.*

Specimens examined:

New York: East Berne, *C. H. Peck* (in Coll. N. Y. State).

3. **C. galeata** Schum. ex Fries, Epicr. 567. 1836–1838.

Plate 19. fig. 2.

Merulius galeatus Schum. Plant. Sællandiæ **2**: 371. 1803.—*Cantharellus galeatus* Fries, Syst. Myc. **1**: 524. 1821; Flor. Dan. **12**: fasc. 34. 11. *pl. 2027. f. 1.* 1830.

Illustrations: Flor. Dan. *pl. 2027. f. 1.*

Fructifications membranaceous-soft, somewhat sessile, obversely cup-shaped and then dimidiate, helmet-shaped, even, whitish, the margin entire; hymenium at length rufescent, slightly wrinkled; spores ovate or obovate, 10 x 8 μ.

Fructifications 4–15 mm. in diameter.

On mosses. Ohio.

When young entire, cup-shaped; gray when moist, snow-white when dry, then rufescent. The above description is that given in European works. The species has been reported from Ohio by Morgan but I have not studied his specimens nor any European specimens of this species. The form and coloration of the pileus and the large spores should distinguish *C. galeata* from the other species which occur on mosses in North America.

4. **C. muscigena** Pers. ex Fries, Epicr. 567. 1836–1838.

Plate 19. fig. 3.

Thelephora muscigena Pers. Syn. Fung. 572. 1801; Fries, Syst. Myc. **1**:524. 1821.—*T. vulgaris* α *candida* Pers. Myc. Eur. **1**:115. *pl. 7. f. 6.* 1822.—*Cantharellus lævis* Fries, Syst. Myc. **1**:524. 1821; Elenchus Fung. 55. 1828.—*Craterellus Pogonati* Peck, Bull. Torr. Bot. Club **33**: 218. 1906.

Illustrations: Persoon, Myc. Eur. **1**: *pl. 7. f. 6.*—Patouillard, Tab. Anal. Fung. *f. 465.*—Oudemans, Ned. Kruidk. Archief III. **2**: *pl. 11. f. 2.*

Pileus membranaceous-soft, sessile, stipitate or attached by upper surface, irregular, flattened, white, externally minutely tomentulose or silky under a lens; stem when present lateral or eccentric, slender, white; hymenium even or sometimes rugulose, drying pinkish buff; spores white in collection on slide, even, apiculate at base, flattened on one side, $4\frac{1}{2}$–5 x $2\frac{1}{2}$–3 μ but only 3–$4\frac{1}{2}$ x 2–3 μ in preparations of the hymenium, borne four to a basidium.

Pileus 2–6 mm. in diameter; stem when present 3–5 mm. long, $\frac{1}{2}$ mm. thick.

On *Polytrichum* and other mosses. New England and New York. August and September.

The fructifications are very variable in form and they are attached in various ways to the moss plants; they may be somewhat incrusting but at some distance above the ground. The substance of the pileus is very soft and its upper surface is somewhat bibulous and shows its interwoven fibers under a lens. The spores of this species are given in Saccardo's 'Sylloge' as 8–10 x 5 μ, but the European specimens of exsiccati cited below have small spores of the dimensions which I give for American specimens, and Bresadola, Ann. Myc. 1: 111. 1903, gives the spore dimensions as 3–4 x 3 μ. The specimens of *C. Pogonati* were described as sterile by Peck; I find them to be rather immature but bearing spores 3 x 2 μ.

Specimens examined:

Exsiccati: Karsten, Fung. Fenn., 441; Krieger, Fung. Sax., 1564.
Finland: *Karsten* (in Herb. Fries), and Fung. Fenn., 441.
Germany: Saxony, *W. Krieger*, Krieger, Fung. Sax., 1564.
Vermont: near Falls of Lana, Salisbury, *E. A. Burt.*
Connecticut: South Windsor, *C. C. Hanmer, 1956*, the type collection of *Craterellus Pogonati* Pk.
New York: Floodwood, *E. A. Burt.*

5. C. arachnoidea Peck, Rep. N. Y. State Mus. 44: 134 (22). 1891.

Type: in Collection New York State.

Fructifications membranaceous, very thin, tender, white, externally downy, irregularly cup-shaped; hymenium somewhat uneven in large specimens; spores colorless, even, somewhat flattened on one side, 4–5 x 3½–4 μ, borne at least two to a basidium.

Fructifications 2–4 mm. in diameter.

On bark and mosses. Vermont and New York. September.

The cups are seated upon or developing from fine, white, loosely branching, webby strings of mycelium. This is a marked character in the type and is the chief character for separating this species from *C. muscigena*. The spores are slightly more globose than in the latter and it may be that the hymenium of *C. arachnoidea* is superior; in *C. muscigena* it is inferior. The hyphæ are about 2 μ in diameter in each species.

Specimens examined:

Vermont: South Lincoln Notch, near Middlebury, *E. A. Burt.*

New York: Carrollton, *C. H. Peck*, type (in Coll. N. Y. State).

6. C. Tiliæ Peck ex Cooke, Grevillea **20:** 9. 1891.

Plate 19. fig. 16.

Peziza Tiliæ Peck, Rep. N. Y. State Mus. **24:** 96. 1872.—*Trichopeziza Tiliæ* (Peck) Sacc. Syll. Fung. **8:** 428. 1889; Seaver, Proc. Iowa Acad. Sci. **12:** 116. 1905; Mycologia **1:** 110. 1909.

Type: in Collection New York State and a portion from it in Kew Herbarium.

Fructifications gregarious, rather fleshy, minute, sessile or nearly so but with firm base, white, globose, then expanded and concave, drying cup-shaped, densely white villose; hairs straight, cylindric, granular incrusted, 200 x 6 μ; hymenium concave, even, ivory-yellow to vinaceous buff; spores white in a collection on a slide, simple, even, ovate, somewhat curved, 12–18 x 6–6½ μ, borne four to a basidium.

Fructifications ½–1 mm. high, ⅓–1 mm. broad; stem, when present, about one-half the height of the whole fructification.

On bark of dead branches of *Tilia Americana* and *Ulmus* on the ground. Canada and Vermont westward to Missouri. March to October. Probably common.

C. Tiliæ has somewhat the habit of *C. albo-violascens* but differs from the latter in having no violaceous tints, in being more hairy, in having slenderer spores, and in having at the base a very firm tubercle which offers considerable resistance when the fructification is crushed under a cover glass or sectioned. While not cespitose the fructifications of *C. Tiliæ* are often so near together that seven or eight have been counted on an area a centimeter square. I refer to *C. Tiliæ* many American specimens which have been distributed under the name *C. pezizoides* Zopf. The European specimens which Sydow has distributed under the latter name seem to me from the studies and comparisons which I made in Kew Herbarium to be *C. Curreyi* B. & Br. rather than *C. Tiliæ.*

Specimens examined:

Exsiccati: Shear, N. Y. Fungi, 55; Ell. & Ev., N. Am. Fungi, 2316a, under the name *C. pezizoides;* Ell. & Ev., Fung.

Col., 5, under the name *C. pezizoides;* Rabenhorst, Fung. Eur., 3942, under the name *C. pezizoides.*

Quebec: Hull, *J. Macoun, 672.*

Ontario: Ottawa, *J. Macoun, 318, 430;* London, *J. Dearness,* Ell. & Ev., N. Am. Fungi, 2316a, and Fung. Col., 5.

Vermont: Middlebury, *C. O. Smith,* and also *E. A. Burt.*

New York: Knowersville (Altamont), *C. H. Peck,* type (portion in Kew Herb.); Alcove, *C. L. Shear,* Shear, N. Y. Fungi, 55.

Ohio: Oberlin, *F. D. Kelsey* (in Mo. Bot. Gard. Herb., 4942).

Michigan: Agricultural College, *G. H. Hicks,* comm. by W. G. Farlow, 6 (in Mo. Bot. Gard. Herb., 43807).

Wisconsin: Blue Mounds, *I. E. Melhus,* comm. by C. J. Humphrey, 2410 (in Mo. Bot. Gard. Herb.).

Missouri: *C. H. Demetrio,* Rabenhorst, Fung. Eur., 3942.

7. C. villosa Pers. ex Karsten, (Mycol. Fenn. 3) Bidrag Finska Vet.-Soc. **25**: 325. 1876. Plate 19. fig. 13.

Peziza villosa Pers. Syn. Fung. 655. 1801; Fries, Syst. Myc. **2**: 104, pr. p. 1823.—An *Cyphella pezizoides* Zopf, in Morgan, (Myc. Fl. Miami Val.) Jour. Cincinnati Soc. Nat. Hist. **10**: 202. 1888?

Illustrations: Patouillard, Tab. Anal. Fung. *f. 257.*

Fructifications gregarious, membranaceous, sessile, drying globose or obconic and with the pore nearly closed by the hairs, white, externally white-villose; the hairs granular incrusted, cylindric, 200 x 5–6 μ; hymenium even, concave; spores hyaline, even, ovoid, flattened on one side, broadest near the base, 10–12 x 5–7 μ.

Fructifications about $\frac{1}{5}$ mm. high, $\frac{1}{5}$–$\frac{1}{4}$ mm. broad.

On dead stems of *Artemisia, Helianthus,* and *Solidago.* South Carolina, Missouri and California. June and July.

The fructifications of *C. villosa* resemble those of *C. Tiliæ* in form, color, and hairiness but are much smaller than those of *C. Tiliæ,* more membranaceous and easily crushed under a cover glass, and have smaller spores. The hymenium is very pale with not more than a very slight yellowish tint.

Specimens examined:

Exsiccati: Krieger, Fung. Sax., 1457; Ravenel, Fung. Am., 459; Ell. & Ev., N. Am. Fungi, 2316b, under the name *Cyphella pezizoides* Zopf.

South Carolina: Aiken, *Ravenel,* Ravenel, Fung. Am., 459.
Missouri: Emma, *C. H. Demetrio,* Ell. & Ev., N. Am. Fungi, 2316b.
California: Half-moon Bay, San Mateo Co., *E. B. Copeland,* Baker, Pacific Coast Fungi, 3611 (in Mo. Bot. Gard. Herb., 4944).

8. C. caricina Peck, Rep. N. Y. State Mus. **33**: 22. 1880.
Plate 19. fig. 8.

Type: in Collection New York State.

Fructifications scattered, membranaceous, sessile, wholly white, externally minutely webby-hairy; hymenium glabrous, uneven in large specimens; basidia cylindric, 20 x 5 μ, 4-spored; spores colorless, even, lanceolate or subclavate, pointed at base, 8–13 x 4 μ.

Fructifications 1–2 mm. broad.

On culms and leaves of carices. New York. August.

The spores of the type are noteworthy by their tapering base.

Specimens examined:
New York: Verona, *C. H. Peck,* type (in Coll. N. Y. State).

9. C. capula Holmsk. ex Fries, Epicr. 568. 1836–1838.
Plate 19. fig. 4.

Peziza Capula Holmsk. Nov. Act. Havn. **1**: 286. *f. 7;* Fung. Dan. **2**: 41. *pl. 22.* 1899.

Illustrations: Holmskiold, Nov. Act. Havn. **1**: 286. *f. 7;* Fung. Dan. **2**: *pl. 22.*—Flor. Dan. **33**: *pl. 1970. f. 3.*—Patouillard, Tab. Anal. Fung. **1**: *f. 35.*

Fructifications membranaceous, obliquely campanulate, extended into an oblique stem, glabrous, whitish, the margin sinuate, irregularly shaped; hymenium even. . . . On dead stems of herbaceous plants.
—Translation of description in Fries' 'Epicrisis.'

Fructifications in the figures of Holmskiold 4–9 mm. high; pileus 2–7 mm. long, 2–4 mm. broad; stem 1–2 mm. long.

On dead stems of *Fœniculum* and other herbs. New York and South Carolina.

I have not been able to study any European specimens of this species. In the copy of Cooke's 'Fungi Britannici' in the herbarium of the Missouri Botanical Garden the packet labeled *C. capula,* 112, contains only some pieces of stubble. The Amer-

ican specimens distributed in Ravenel's 'Fungi Americani,' 458, were determined by Cooke. In their present dried condition these specimens agree well with Holmskiold's illustrations in form; the stem of these specimens is now hair-brown and the pileus pale olive-buff; their dimensions are: fructifications 1–3 mm. long, pileus ½–2 mm. long and broad; stem ⅓–1 mm. long x 100 μ thick. The basidia are 16–20 x 3½–4½ μ; spores colorless, even, flattened on one side, 4½–6 x 3–3½ μ.

Specimens examined:

Exsiccati: Ravenel, Fung. Am., 458.

South Carolina: Aiken, *Ravenel*, Ravenel, Fung. Am., 458.

10. C. minutissima Burt, n. sp. Plate 19. fig. 5.

Type: in Mo. Bot. Gard. Herb. and in Farlow Herb.

Fructifications gregarious, very minute, membranaceous and very delicate, sessile, globose, snow-white, externally villose, often with mouth oblique, margin inrolled; hairs white, incrusted, 75–90 x 4 μ; hymenium concave, white; basidia clavate, 16 x 4 μ; spores colorless, even, 5–6 x 4–4½ μ.

Fructifications 200–500 μ broad, about 200–500 μ high.

On inner bark of *Populus*. New Hampshire. August.

The characters of this species agree in some details with those in the incomplete description of *C. globosa* Pat., the specimens of which were collected on the under side of leaves of ferns in Ecuador by von Lagerheim, but as no mention is made of spore characters for *C. globosa* and as other species of *Cyphella* have not been found to vary widely with regard to kind of substratum, it seems best to regard our New England species as probably distinct. *C. punctiformis* (Fries) Karst. is a small white *Cyphella*, described by Karsten as having spores 5–8 x 2–4 μ; I have not been able to study authentic specimens of *C. punctiformis*, but comparison of *C. minutissima* with this species of northern Europe should be made.

I refer to *C. minutissima* a collection made by myself in Vermont on bark of rotting locust limbs. The fructifications of this collection lack spores but agree in all other respects with the type.

Specimens examined:

New Hampshire: Chocorua, *W. G. Farlow, 3*, type (in Mo. Bot. Gard. Herb., 43803, and in Farlow Herb.).

Vermont: Middlebury, *E. A. Burt.*

11. C. Langloisii Burt, n. sp. Plate 19. fig. 6.

Type: in Farlow Herb. and Burt Herb.

Fructifications gregarious, membranaceous, cup-shaped, sessile, drying pale ivory-yellow, externally downy pubescent, the margin inrolled; hairs colorless, somewhat crinkled together, granular incrusted, 100–150 x $3\frac{1}{2}$–$4\frac{1}{2}$ μ; hymenium concave, even, pale ivory-yellow to cream color; spores colorless, even, pointed at the base, 4–7 x 3–4 μ; basidia clavate, 20 x 5μ, 2-spored.

Fructifications about $\frac{1}{4}$ mm. high; $\frac{1}{4}$–$\frac{1}{2}$ mm. broad.

On dead stems of *Arundinaria* and on decaying pieces of wood lying on the ground. Louisiana. September and April.

The fructifications of *C. Langloisii* are about as small as those of *C. minutissima* but differ from them in being somewhat extended laterally and occasionally somewhat laterally confluent rather than always globose, in having an ivory-yellow rather than snow-white color, and in having the hymenium colored and the hairs longer than in *C. minutissima*. Comparison should be made with *C. fraxinicola* B. & Br., of which I have studied no specimens but which seems distinct by some characters of the incomplete published description.

Specimens examined:

Louisiana: St. Martinville, *A. B. Langlois, 1802*, type (in Farlow Herb.), and *cz*, type, in Burt Herb., and *cy*, and from the same collector but comm. by W. G. Farlow, 5 (in Mo. Bot. Gard. Herb., 43791).

12. C. porrigens Burt, n. sp. Plate 19. fig. 7.

Type: in Burt Herb. and New York Bot. Gard. Herb.

Fructifications scattered, membranaceous, thin, wholly cream-color, sessile, obversely cup-shaped or helmet-shaped, resupinate by the upper surface of one side but with the greater portion of the pileus extended and reflexed; hymenium inferior, somewhat wrinkled when moistened, concave, basidia clavate, 20–25 x 4–$4\frac{1}{2}$ μ, with four sterigmata; spores colorless, even, flattened on one side, obovate, $7\frac{1}{2}$ x $4\frac{1}{2}$ μ.

Fructifications $\frac{1}{2}$–1 mm. broad.

On dead prickle-bearing stems, possibly *Rubus sp.* Wet mountainous region at altitude 4500–5200 feet. Cinchona, Jamaica. About January 1.

This species does not appear closely related to any other

species; it is marked by the resupinate-reflexed habit of most fructifications; only rarely is a fructification attached by its vertex. The dried specimens are externally minutely fibrillose under a lens but do not show hairs in microscopic preparations. When the fructifications are moistened the hymenium shows two or three minute wrinkles radiating from an eccentric point.

Specimens examined:

Jamaica: Cinchona, *W. A. and Edna L. Murrill,* N. Y. Bot. Gard., Fungi of Jamaica, 607, type.

13. C. cupulæformis Berk. & Rav. Grevillea 2: 5. 1873. Plate 19. fig. 9.

Type: type and cotype in Kew Herb. and in Curtis Herb. respectively.

Fructifications scattered, rarely in clusters of two or three, sessile, cup-shaped, somewhat globose, externally mineral gray and obscurely tomentose, the margin incurved; hymenium concave, even, fuscous; basidia clavate, 20–25 x 4–6 μ, having 2–4 sterigmata which become finely attenuated; spores colorless, angular, $4\frac{1}{2}$–6 x $4\frac{1}{2}$ μ.

Fructifications $\frac{1}{2}$ mm. high, $\frac{1}{2}$–1 mm. broad.

On bark of *Juniperus virginiana.* South Carolina and Georgia.

The hairiness of the exterior of the pileus is due to the irregularly curved and interwoven hyphæ which form the surface layer of the pileus; these hyphæ are colorless and about 3 μ in diameter, and they bear scattered but large incrusting granules. The angular spores of this species are often octahedral in form and are noteworthy for *Cyphella;* at maturity, they are attached to the basidium by sterigmata becoming 6 μ long and so finely attenuated that the attachment of the spores to the basidia is made out with difficulty. This species may be readily known by its occurrence on bark of *Juniperus virginiana* and by its angular spores.

Specimens examined:

Exsiccati: Ravenel, Fung. Am., 224.

South Carolina: *Ravenel, 1403,* type (in Kew. Herb.).

Georgia: Darien, *Ravenel,* Ravenel, Fung. Am., 224.

14. C. griseo-pallida Weinm. Hymeno- et Gastero-mycetes in Rossico. 522. 1836.

Illustrations: Patouillard, Tab. Anal. Fung. *f. 255.*

Fructifications gregarious, adnate-sessile, membranaceous, wholly gray-pallid, externally flocculose; hymenium glabrous, even.

At first having the form of globose, closed granules, soon open, campanulate or crateriform, often dimidiate in old stages.

Fructifications ½ mm. high, ½–2 mm. broad.

On moist ground and on pine wood thinly covered with earth and on old cracked trunks of *Lonicera tartarica* (in Europe). —Translation of original description.

On bark, twigs and leaves lying on the ground. New York and Ohio. November.

I have not seen the type of *C. griseo-pallida* nor any European specimens which have been compared with it, but Peck, Rep. N. Y. State Mus. **30**: 48. 1879, has referred to this species a collection which he made at Sand Lake, New York. Peck notes that his specimens sometimes have a very short stem. I found the spores of these specimens hyaline, even, somewhat flattened on one side, 4 x 3 μ; basidia 12 x 4 μ.

Specimens examined:

New York: Sand Lake, *C. H. Peck* (in Coll. N. Y. State).

15. C. subgelatinosa Berk. & Rav. Grevillea **2**: 5. 1873.

Type: in Kew Herb.

Fructifications scattered, somewhat gelatinous, sessile, flattened, externally cinereous and farinaceous, the thin margin inflexed; hymenium slightly convex, even, brown; basidia clavate, about 25 x 5–6 μ, probably 2-spored; spores colorless, even, ellipsoidal, 8 x 3½ μ.

Fructifications about 1½ mm. broad.

On *Alnus serrulata*. South Carolina.

The fructifications of the type have dried with the slightly convex hymenium so prominently visible that they resemble brown apothecia of lichens with a pale margin (exciple). The most of the basidia are immature; I found one showing two sterigmata distinctly. No spores were found attached to basidia; the spore characters, which are given above, are those of loose spores in the preparation. *C. subgelatinosa* is so very distinct from our other species of *Cyphella* that it will probably be overlooked by botanists collecting *Basidiomycetes* only, unless especially kept in mind.

Specimens examined:

South Carolina: Aiken, *Ravenel, 1714*, type (in Kew Herb.).

16. C. Ravenelii Berk. Grevillea **2**: 5. 1873. Plate 19. fig. 14.

Type: type and cotype in Kew Herb. and in Curtis Herb. respectively.

Fructifications single or gregarious, sessile, subglobose, somewhat flattened, depressed at the pore, minutely hairy under a lens, vinaceous buff; hairs minutely rough, about 300 μ long, 4 μ thick, tapering towards the free end, olive-yellow under the microscope; spores hyaline, or perhaps very slightly colored, even, broadly ellipsoidal, 10–12 x 6–8 μ.

Fructifications 0.6 mm. high, 0.8 mm. broad; pore 0.15 mm. in diameter.

On bark of *Carya*. South Carolina.

The specimens of this species which I have seen have been on thick and cracked portions of bark apparently from large branches or the main trunk of the tree. Sometimes only one fructification occurs on a piece of bark a centimeter square; sometimes such a piece bears from 3 to 6 fructifications with some of them barely in contact with one another. The type specimen contains so few fructifications that I made a microscopic preparation at Kew Herbarium from the specimen distributed by Ravenel in Ellis, N. Am. Fungi, 721, which seems to me to be certainly the same species as the type. Berkeley described the spores in his original description as "elliptic, .00025 (in.) long"; I found them about twice this length in my preparation referred to and also in a preparation recently made from the specimen in Ravenel, Fung. Am., 130, in the Mo. Bot. Gard. Herb.

Specimens examined:

Exsiccati: Ravenel, Fung. Am., 130; Ellis, N. Am. Fungi, 721.

South Carolina: Aiken, *Ravenel, 1755*, the type and cotype (in Kew Herb. and in Curtis Herb. respectively); and also Aiken, *Ravenel*, Ravenel, Fung. Am., 130, and Ellis, N. Am. Fungi, 721.

17. C. texensis Berk. & Curtis, Grevillea **20**: 9. 1891. Plate 19. fig. 10.

Type: in Kew Herb.

Fructifications scattered, sessile, pallid but at present time

Isabella-color (melleus of 'Chromotaxia'), cup-shaped, at length flattened and disk-shaped, externally hairy; hairs olive-ocher under the microscope, granular incrusted, cylindric, 300–400 x 4½–6 μ; basidia clavate, 25–30 x 6–8 μ, 4-spored; spores hyaline, even, broadly ellipsoidal, 13 x 8 μ.

Fructifications 1–1½ mm. broad.

On *Quercus*. Texas.

The type is scanty, consisting of three fructifications, but these fructifications are in fine condition and present well the characters of the species. *C. texensis* now impresses me as more closely related to *C. Ravenelii* than I observed when studying the specimens of both in Kew Herbarium. The fructifications of *C. texensis* are the melleus of Saccardo's 'Chromotaxia' and the hairs are of a little greater diameter and have larger incrusting granules than those of *C. Ravenelii*, but the spores and basidia are very similar in form and dimensions in both species.

Specimens examined:

Texas: *Wright, 3779*, type (in Kew Herb.).

18. C. mellea Burt, n. sp. Plate 19. fig. 12.

Type: in Burt Herb. and in U. S. Dept. Ag. Herb.

Fructifications closely gregarious, sessile, Isabella-color, spherical and with margin inrolled in the dried state, sometimes obconic, externally hairy; hairs granular incrusted, baryta-yellow under the microscope, cylindric, 80–100 x 3½–4 μ; hymenium even, whitish or pale olive-buff; basidia clavate, 12–16 x 6 μ; spores mostly colorless but some pale baryta-yellow, even, broadly ellipsoidal, 5–6 x 4–4½ μ.

Fructifications about ⅕–½ mm. high and broad.

On rotten wood of *Salix nigra*. Louisiana. December.

In the specimen upon which the description is based, the most of the fructifications are about ⅕ mm. high and broad and are distributed on the rotten wood at the rate of about 200 per square centimeter. Rarely a short stem-like base is visible when the fructifications emerge from the bottom of small crevices between the fibers of the wood, but the fructifications are generally sessile. The species is intermediate between *Cyphella* and *Solenia* but is included in the former genus because the fructifications do not arise from a common subiculum and are more globose than in *Solenia*. The description of *C. mellea* suggests

those of *C. Ravenelii* and *C. texensis* in many respects, but the fructifications are much smaller and more numerous than in either of these species, and their various parts are also much smaller and some of the spores are colored.

Specimens examined:

Louisiana: Bohemia, Plaquemines Co., *A. B. Langlois, 864a*, type, in Burt Herb. and also (in U. S. Dept. Ag. Herb.); *A. B. Langlois, 864* (in U. S. Dept. Ag. Herb.).

19. C. fasciculata Schw. ex Berk. & Curtis, Jour. Acad. Nat. Sci. Phila. **3**: 207. 1856. Plate 19. fig. 17.

Cantharellus fasciculatus Schw. Trans. Am. Phil. Soc. N. S. **4**: 153. 1831.—*C. fasciculatus* Schw. in Saccardo, Syll. Fung. **5**: 495. 1887.—*Cyphella fasciculata* Berk. & Curtis, Grevillea **2**: 6. 1873.—*Solenia anomala* Pers. var. *orbicularis* Peck, Rep. N.Y. State Mus. **47**: 168 (42). 1894.—*Cyphella fulva* Berk. & Rav. Grevillea **2**: 5. 1873.—*C. Ravenelii* Saccardo, Syll. Fung. **6**: 672. 1888.—*C. Saccardoi* Sydow, in Saccardo, Syll. Fung. **14**: 233. 1900.—*C. furcata* Berk. & Curtis, Grevillea **2**: 5. 1873.

Type: in Herb. Schweinitz.

Fructifications gregarious, sometimes fascicled, pezizoid, tawny olive; pileus stipitate, cup-shaped, extended vertically or pendulous, tomentose with tawny-olive, even-walled hairs which are flexuous or somewhat spirally curved towards the tips, the margin strongly inrolled; stem short, variable in length, cylindric, tomentose, colored like the pileus; hymenium concave, even, drying olive-buff; spores hyaline, even, cylindric, slightly curved, 7–9 x 2–2½ μ, borne four to a basidium.

Fasciculate clusters about 2 mm. in diameter, 1 mm. high; fructifications ⅓–1 mm. in diameter, 1–2 mm. high; stem ½–1 mm. long, ⅕–½ mm. thick.

On bark of twigs of *Alnus* in swamps and rarely on *Prunus virginiana* and *Pyrus Malus*. Canada and Newfoundland to South Carolina and westward to Wisconsin. Throughout the year, more highly fasciculate from autumn to spring. Common.

This fungus is very common on dead twigs of *Alnus* in swamps. The color is similar to that of *Solenia anomala* but the fructifications are rather larger and more cup-shaped than those of the latter and have the hymenium merely concave rather than lining a tube. The fructifications burst out through the outer bark

either singly or in clusters of from two to twenty individuals more or less connected together at the base. The differences in habit between the extremes of highly fascicled forms and those with fructifications gregarious and largely single, impress one as of specific weight at first and I should like to recognize these extremes as two species but they intergrade too completely. The dated collections which I have seen, indicate that the specimens become highly fasciculate in autumn and winter.

I do not understand why Berkeley attempted authorship for this species. The *C. fasciculata* B. & C. is certainly that of Schweinitz both in description and in fascicled form of types; and as for *C. fulva* B. & Rav., it is noted in the original description that it is the same as *Cantharellus fasciculatus* Schw.

Specimens examined:

Exsiccati: Ellis, N. Am. Fungi, 936, fascicled form; Ell. & Ev., Fung. Col., 1818, fascicled form under the name *C. Ravenelii* Berk.; Shear, N. Y. Fungi, 308, fascicled form under the name *Solenia anomala* (Pers.) Fr. var. *orbicularis*. Pk. Peck det.; Ravenel, Fung. Car. IV., 16, the type distribution of *C. fulva* B. & Rav.; Ravenel, Fung. Am., 129 (bearing spores in abundance); Shear, N. Y. Fungi, 56.

Newfoundland: Headquarters, *B. L. Robinson & H. von Schrenk* (in Mo. Bot. Gard. Herb., 4764 and 43789, the latter communicated by W. G. Farlow); Bay of Islands, *A. C. Waghorne, 127* (in Mo. Bot. Gard. Herb., 42593).

Quebec: Hull, *J. Macoun, 355.*

Ontario: Ottawa, *J. Macoun, 23.*

Maine: *J. Blake* (in Curtis Herb., 6926, and in Kew Herb.).

New Hampshire: Conway, *W. G. Farlow;* North Conway, *W. G. Farlow* (in Mo. Bot. Gard. Herb., 43786); Shelburne, *H. von Schrenk* (in Mo. Bot. Gard. Herb., 4765), *W. G. Farlow* (in Mo. Bot. Gard. Herb., 43787); Franklin Falls, *Mrs. J. B. Harrison,* Ellis, N. Am. Fungi, 936.

Vermont: Middlebury, on *Alnus* and on *Prunus virginiana*, *E. A. Burt.*

Massachusetts: Newton, *W. G. Farlow* (in Mo. Bot. Gard. Herb., 42591, 42592 and 43788).

New York: *Torrey*, type (in Herb. Schw.); *Sartwell*, cotype and type of *C. fasciculata* B. & C. (in Curtis Herb., 2659, and in

Kew Herb. respectively) and specimen (in Mo. Bot. Gard. Herb., 4937); Ithaca, *G. F. Atkinson;* East Galway, *E. A. Burt;* Keeseville, *C. O. Smith*, Ell. & Ev., Fung. Col., 1818; Alcove, *C. L. Shear*, Shear, N. Y. Fungi, 56 and 308; Albany, *C. H. Peck*, comm. by H. D. House (in Mo. Bot. Gard. Herb., 43821); Karner, *C. H. Peck*, comm. by H. D. House (in Mo. Bot. Gard. Herb., 43820).

South Carolina: *Ravenel, 1683* (in Curtis Herb. and in Kew Herb.), and in Ravenel, Fung. Car. IV., 16; Aiken, *Ravenel*, Ravenel, Fung. Am., 129.

Alabama: *Beaumont*, the cotype and type of *C. furcata* (in Curtis Herb., 4022, and in Kew Herb. respectively).

Wisconsin: Madison, *W. Trelease* (in Mo. Bot. Gard. Herb., 42594).

20. C. conglobata Burt, n. sp. Plate 19. fig. 15.

Type: in Mo. Bot. Gard. Herb. and in Farlow Herb.

Fructifications cespitose, 10–30 together, sessile on a common short trunk which is erumpent through the bark; individual fructifications subglobose, fuscous and glabrous when moist, drying mouse-gray and with the margin inrolled; hymenium concave, black or nearly black; basidia simple, with four sterigmata; spores colorless, even, cylindric, slightly curved, 8–10 x $2\frac{1}{2}$–3μ.

Cluster 1–2 mm. in diameter, emerging about $\frac{1}{2}$ mm. from the bark; cups 400–500 μ broad, nearly as high.

Clusters scattered on small limbs of *Alnus*. New Hampshire and New York. July and September.

The clusters of this curious fungus are distributed at the rate of about 5 or 6 clusters to the square centimeter on what I conclude to have been the under side of a horizontal limb—perhaps a limb prostrate on the ground; for cups in clusters exactly on this presumably under side have the pore central while in the clusters which emerged more obliquely from the limb the cups are somewhat auriform with oblique pore and are arranged in imbricated manner. The outer surface of the cups is composed of irregularly branched and interwoven pale brownish hyphæ about 2 μ in diameter. The substance of the fructifications and common trunk-like base is composed of colorless hyphæ with walls gelatinously modified.

One might regard this fungus as the type species of a new genus distinct from *Cyphella* or *Solenia* by the common central mass on which the individual cups are borne, but in *Cyphella fasciculata* the cups sometimes occur singly and sometimes branching from a common central or basal mass. For this reason it seems best to include the present species in *Cyphella* through its relationship in plan of structure to *C. fasciculata*, from which it is specifically distinct in other respects, however. Both these species are excluded from *Solenia* by their short and globose fructifications and by the absence of a subiculum on the general area over which the clustered fructifications are distributed.

Specimens examined:

New Hampshire: Lower Bartlett, *R. Thaxter*, comm. by W. G. Farlow, 4, type (in Mo. Bot. Gard. Herb., 43806, and in Farlow Herb.).

New York: Adirondack Mts., *C. H. Peck*, comm. by H. D. House (in Coll. N. Y. State and in Mo. Bot. Gard. Herb., 43818); North Elba, *C. H. Peck*, comm. by H. D. House (in Mo. Bot. Gard. Herb., 43819).

21. C. fumosa Cooke, Grevillea **20**: 9. 1891. Plate 19. fig. 11.

Type: in Kew Herb.

Fructifications gregarious, membranaceous, cup-shaped, flexuous, sepia or olive-brown and blackening, even, attenuated below into a very short stipe, or sessile; hymenium even; basidia cylindric-clavate, 20 x 4–5 μ; spores colorless, even, somewhat flattened on one side, 6–8 x 3½–4 μ.

Fructifications 1–2 mm. broad.

On rotting leaves of *Gladiolus*. South Carolina.

Cooke described the spores of this species as globose, 4 μ in diameter, but I found no such spores in my preparation from the type. Spores 6–8 x 3½–4 μ are abundant and are probably the spores of this species, although I could not find any spores still attached to the basidia. I conclude from my microscopical preparations that the fructifications are glabrous.

Specimens examined:

South Carolina: Aiken, *Ravenel, 3071*, type (in Kew Herb.).

SPECIES IMPERFECTLY KNOWN

C. cinereo-fusca Schw. ex Saccardo, Michelia **2**: 303. 1881.

Peziza cinereo-fusca Schw. Schrift. d. Naturforsch. Gesell., Leipzig, **1**: 119. 1822; Fries, Syst. Myc. **2**: 97. 1823.—*Cyphella cinereo-fusca* (Schw.) Sacc. Syll. Fung. **5**: 674. 1888.—*Lachnella cinereo-fusca* (Schw.) Sacc. Syll. Fung. **8**: 399. 1889.

Fructifications minute, gregarious, sessile, externally farinaceous-hirsute and ash-green, the margin incurved; hymenium fuscous-bay.

On decorticated branches of *Cercis*. [North Carolina.]

3 mm. broad. Cups often closed.

—Translation of original description.

I have not seen an authentic specimen of this species nor anything on *Cercis* which seems referable to it. The species is given here on the authority of Saccardo, *l. c.*, who refers to this species a *Cyphella* collected on *Vitis vinifera* near Toulouse, France, by Roumeguere. Saccardo does not state that he made comparison with an authentic specimen from Schweinitz, and he has entered the species in the 'Sylloge Fungorum' in both the *Basidiomycetes* and the *Discomycetes*.

C. Palmarum Berk. & Curtis, (Fung. Cub.) Jour. Linn. Soc. Bot. **10**: 337. 1867.

Type: type and cotype probably in Kew Herb. and Curtis Herb. respectively.

White, pileus cyathiform, externally obscurely pruinose; stem short, tomentose, rather thick.

Scarcely 2 mm. high; stem rather thick for the size of the pileus, often oblique.

On petioles of palms. Cuba. June. *C. Wright, 753.*

—Arranged from original description.

C. Peckii Sacc. Syll. Fung. **6**: 684. 1888.

C. candida Peck, Rep. N. Y. State Mus. **27**: 99. 1875.

Type: in Coll. N. Y. State.

Fructifications scattered or gregarious, membranaceous, soft, obconic, nearly or quite sessile, sometimes deflexed, wholly white, externally tomentose; hairs tapering to a sharp point, rough-walled, 60–70 x $3\frac{1}{2}$ μ.

Fructifications about 1 mm. broad.

On dead stems of ferns, *Osmunda cinnamomea*. New York. September.

The type specimens of this species are immature. I could make out neither distinct asci nor basidia in the hymenium. In a crushed preparation I found one spore, colorless, even, pointed at one end, 6 x 2½ μ. It may have been a basidiospore of this species or it may have been a foreign spore.

Specimens examined:

New York: Forestburgh, *C. H. Peck*, type (in Coll. N. Y. State).

C. perexigua Sacc. Michelia **2**: 136. 1880.

Cups bell-shaped, very short and obliquely stipitate, small, ½–¾ mm. long, thin-membranaceous, internally and externally whitish cinereous, externally minutely puberulent; spores not seen. Appears related to *C. erucæformis* and *cupuliformis* but is one-third as large. . . . On decorticated branches. South Carolina. *Ravenel.*—Translation of original description.

I have not seen the type of *C. perexigua*, which is probably in Saccardo Herb. As basidia and basidiospores have not been found for American specimens, it is uncertain whether this species is a *Cyphella*. Patouillard, Tab. Anal. Fung. 19. *f. 34.* 1883, referred to *C. perexigua* a species of *Cyphella* which he collected at Poligny, France, but that reference is doubtful in the absence of knowledge in regard to basidia and basidiospores for American specimens.

C. pezizoides Zopf, in Morgan, (Myc. Fl. Miami Val.) Jour. Cincinnati Soc. Nat. Hist. **10**: 202. 1888.

Type: probably in the State Univ. of Iowa Herb.

"Fructifications membranaceous, nearly sessile, globose then cup-shaped, clothed externally with long erect white hairs. Hymenium even, brownish; spores obovate, .012–.013 mm. in length.

"On old herbaceous stems; not common, cupule pezizoid, scarcely pedicillate, about half a line in diameter. The long hairs are erect and connivent over the hymenium; they are hyaline and incrusted with crystals of calcium oxalate."

—Original description.

The type is not accessible at present.

C. trachychæta Ell. & Ev. Jour. Myc. 4:73. 1888.

Type: in New York Bot. Gard. Herb.

Fructifications gregarious, sessile by a narrow base, white, cup-shaped, clothed outside with appressed hairs; hairs subhyaline, very rough, with a smooth tapering tip 12–15 μ long; hairs paler around the base of the fructification and coarsely roughened by irregularly shaped tubercles, some of which are prolonged into short spines; hymenium nearly white with a slight tinge of slate color; basidia and spores could not be well made out, but the latter are apparently very minute.

Fructifications 300–400μ high and broad, occasionally 1 mm. broad and with the margin distinctly lobed.

On fallen leaves of *Quercus*. Louisiana. July.

The above description is arranged from that originally published. I am under obligation to Dr. Murrill for recently sending to me a portion of the type for study, but the specimen proves too immature to show whether this species is a basidiomycete. The hymenium of this specimen is now pale olive-buff; the hairs are 50–75 x 6 μ, heavily encrusted except near the tips, but I failed to find any hairs roughened by tubercles or bearing spines.

Specimens examined:

Louisiana: *A. B. Langlois, 1424*, type (in N. Y. Bot. Gard. Herb.).

C. Bananæ Cooke, Grevillea **6:** 132. 1878.

Type: probably in Kew Herb.

Fructifications fuliginous or wood-brown, finger-shaped, pendulous-extended behind, glabrous, the margin entire; hymenium white, rugose; spores linear, obtuse, curved, 10–12 x $2\frac{1}{2}$ μ. —Translation of original description.

On dead leaves of *Musa*. Gainesville, Florida. *Ravenel.*

C. filicicola Berk. & Curtis, Grevillea **2:** 5. 1873.

Type: type and cotype probably in Kew Herb. and Curtis Herb. respectively.

Stem very short; cups irregular, sometimes oblique, externally very obscurely tomentose, umber.

On dead fern. North Carolina. Curtis Herb., 4934, type.

The above contains all the items of the original description; I overlooked this species when studying in Curtis Herb. and in Kew Herb.

C. musæcola Berk. & Curtis, Jour. Linn. Soc. Bot. **10**: 337. 1867.

Type: type and cotype in Kew Herb. and Curtis Herb. respectively.

Pileus crucible-form, pallid purple, with very short stem or sessile, externally tomentose; hymenium luteus (cadmium-yellow). —Translation of original description.

About 2 mm. across.

On sheaths of plantain leaves. Cuba. *C. Wright, 751.*

By the kindness of Dr. Farlow I have been permitted to examine a specimen from the type collection. I fail to find any fructifications of a *Cyphella* present. A leaf-spot fungus has caused some dark purple discolorations 1–2 mm. in diameter at various points in the surface of the leaf.

Specimens examined:

Cuba: *C. Wright, 751*, comm. by W. G. Farlow (in Mo. Bot. Gard. Herb., 43790).

EXCLUDED SPECIES

C. convoluta Cooke, (Fungi of Texas) Ann. N. Y. Acad. Sci. **1**: 179. 1878.

Type: In Kew Herb.

"Scattered, cup-shaped, then flattened, 1 to 2 mm. wide, margin membranaceous, involute, externally white, internally fleshy-red; spores oblong (.007 mm. long).

"On trunks. Ravenel (295)."—The original description.

I examined the type of this fungus, which was collected at Houston, Texas, and do not regard it as a *Cyphella*. The "basidia" are filiform and only 1-spored; spores are abundant, hyaline, even, 4–5 x 2–2½ μ.

C. Cupressi Schw. ex Fries, Epicr. 567. 1836–1838.

Merulius Cupressi Schweinitz, Schrift d. Naturforsch. Gesell., Leipzig, **1**: 92. 1822.

This species is an insect gall, not a Basidiomycete. Its true nature seems to have been first pointed out by Berkeley & Curtis, Jour. Acad. Nat. Sci. Phila. **3**: 207. 1856.

C. subcyanea Ell. & Ev. Jour. Myc. **2**: 37. 1885.

As this species is not mentioned in Saccardo's 'Sylloge Fungorum' and as the early numbers of the Journal of Mycology are rare, I quote the original description as follows:

"On living leaves of Sabal Palmetto, Louisiana, Nov. 1885. Rev. A. B. Langlois, No. 57. Shallow cup-shaped, thin, substipitate, oblique, less than 1 mm. across, whitish and nearly smooth outside, hymenium bluish or lead colored. Spores filiform multinucleate, upper end thickened, curved into a semicircle, 40–60 μ long by $1\frac{1}{2}$ μ thick, on short (11–12 x $1\frac{1}{2}$–2 μ) subcylindrical sporophores, which are a little thickened below."

This species was distributed in 1891 in Ell. & Ev., N. Am. Fungi, 2602, the specimens having been collected on living stems of *Smilax* in Louisiana by Mr. Langlois. Mr. Langlois communicated to me still better specimens on dead canes of *Arundinaria*. The fructifications occur scattered here and there in grayish areas 2–4 mm. long by $\frac{1}{2}$–1 mm. broad on the surface of the stems. Dr. Farlow informs me in a letter as the proofs are at hand that the above species is the lichen *Heterothecium Augustinii* Tuckm.

(To be continued.)

Explanation of Plate

PLATE 19

The figures of this plate have been reproduced natural size from photographs of dried herbarium specimens except in the cases noted otherwise.

Fig. 1. *Craterellus borealis.* From the type specimen collected at Gready Island, Labrador, by Owen Bryant.

Fig. 2. *Cyphella galeata.* From photograph, natural size, of the figure in Flor. Dan. *pl. 2027. f. 1.*

Fig. 3. *C. muscigena.* The two figures on the left are from specimens collected at Floodwood, New York, by E. A. Burt; the two on the right are from the type collection of *Craterellus Pogonati* collected at South Windsor, Connecticut, by C. C. Hanmer, 1956.

Fig. 4. *C. capula.* From photograph, natural size, of the figure in Fung. Dan. **2**: *pl. 22.*

Fig. 5. *C. minutissima.* From the type specimens collected at Chocorua, New Hampshire, by W. G. Farlow, 3. Drawings of, *a*, two fructifications, x14; *b*, spores, x510; *c*, a hair from outer wall of fructification, x510.

Fig. 6. *C. Langloisii.* From the type specimens collected at St. Martinville, Louisiana, by A. B. Langlois, cz. Drawings of, *a*, two fructifications, x17; *b*, spores, x510; *c*, a hair from outer wall of fructification, x510.

Fig. 7. *C. porrigens.* From the type specimens collected at Cinchona, Jamaica, by W. A. and Edna L. Murrill, 607. Drawings greatly enlarged of, *a*, a fructification showing attachment to a piece of woody stem; *b*, diagrammatic section of the same fructification; *c*, two spores, x510.

Fig. 8. *C. caricina.* Three spores, x510, from the type specimen collected at Verona, New York, by C. H. Peck.

Fig. 9. *C. cupulæformis.* From the specimens in Ravenel, Fung. Am., 224, collected at Darien, Georgia, by Ravenel. Drawings of, *a*, two fructifications, x6; *b*, a basidium, x510; *c*, four spores, x510.

Fig. 10. *C. texensis.* Three spores, x510, from the type specimens collected in Texas, by C. Wright, 3779.

Fig. 11. *C. fumosa.* Three spores, x510, from the type specimens collected at Aiken, South Carolina, by Ravenel, 3071.

Fig. 12. *C. mellea.* From the type specimens collected at Bohemia, Louisiana, by A. B. Langlois, 864a. Photograph, *a*, of a piece of wood bearing many fructifications, and drawings of, *b*, median longitudinal section of a fructification, x60; *c*, three spores, x510; *d*, a hair from outer wall of fructification, x510.

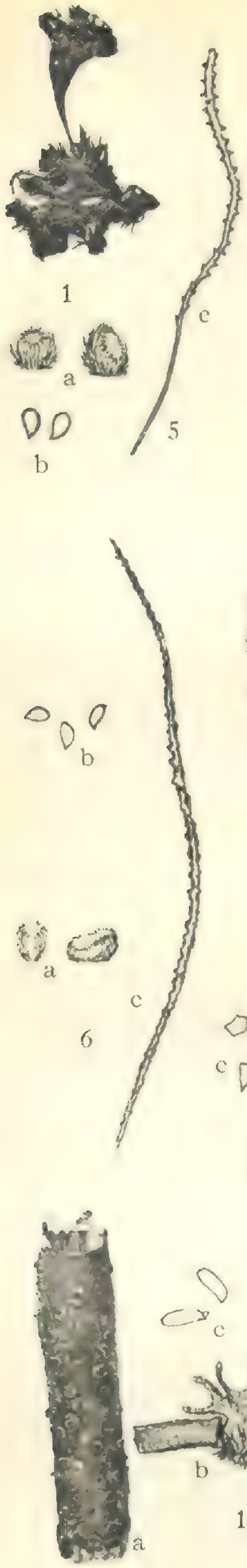
Fig. 13. *C. villosa.* Three spores, x510, from the specimens in Krieger, Fung. Sax., 1457, collected at Königstein, Germany, by W. Krieger.

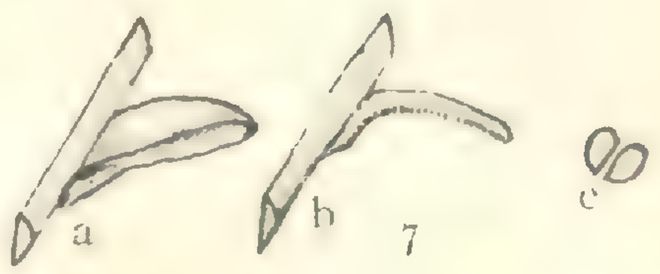
Fig. 14. *C. Ravenelii.* From the specimens in Ravenel, Fung. Am., 130, collected at Aiken, South Carolina, by Ravenel. Drawings of, *a*, a fructification on a piece of bark, x6; *b*, two spores, x510.

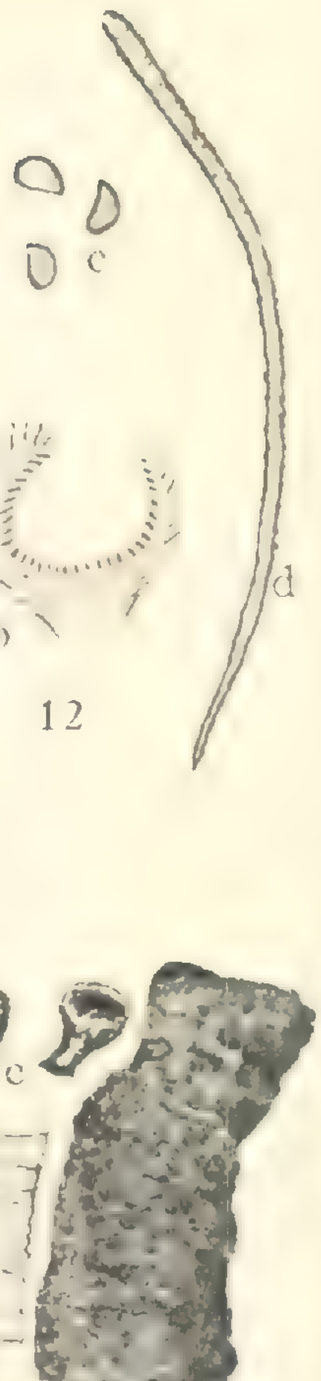
Fig. 15. *C. conglobata.* From the type specimens collected at Lower Bartlett, New Hampshire, by R. Thaxter. Photograph, *a*, of a portion of a branch bearing many clusters of fructifications, and drawings of, *b*, a median vertical section through one cluster of fructifications, x6; *c*, two spores, x510.

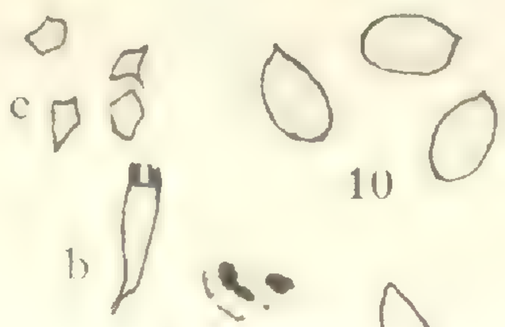
Fig. 16. *C. Tiliæ.* From specimens collected at Middlebury, Vermont, by E. A. Burt. Photograph of, *a*, a piece of limb bearing many fructifications, and drawing of, *b*, three spores, x510.

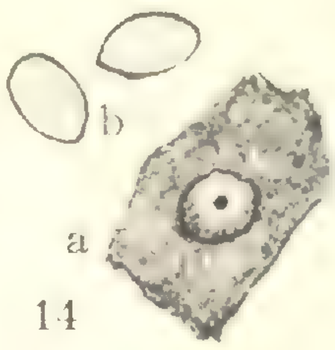
Fig. 17. *C. fasciculata.* From specimens collected at Ottawa, Canada, by J. Macoun, 23. Photograph of, *a*, a piece of bark bearing many fructifications, and drawings of, *b*, a cluster of fructifications, x6; *c*, three fructifications, x10; *d*, two spores, x510.

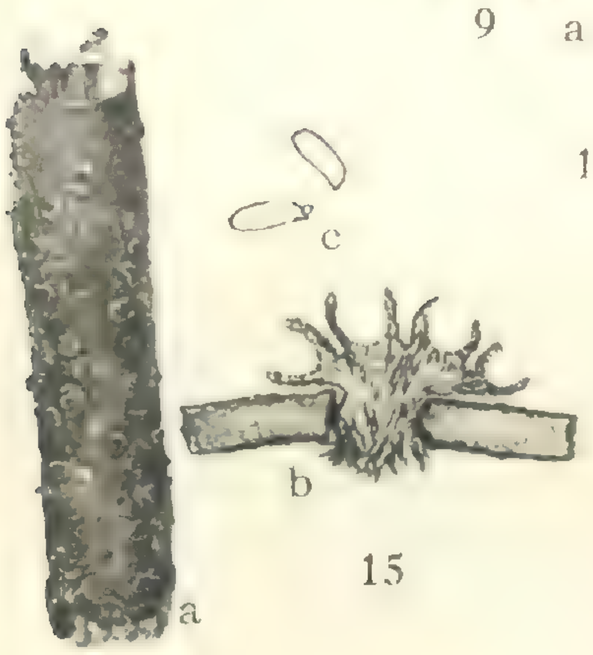


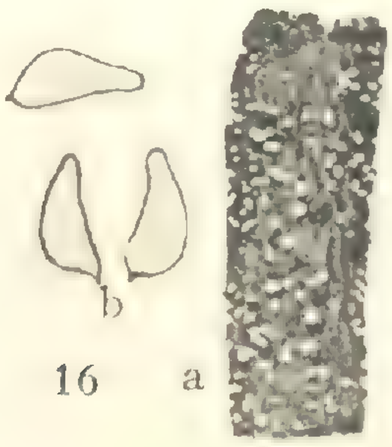


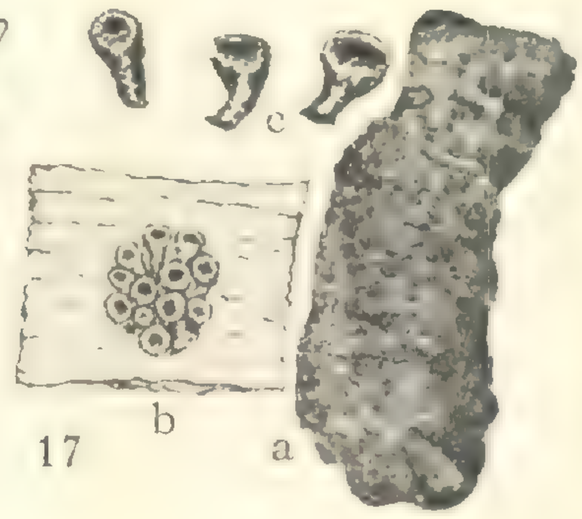












BURT—THELEPHORACEAE OF NORTH AMERICA

1. CRATERELLUS BOREALIS.—2. CYPHELLA GALEATA.—3. C. MUSCIGENA.—4. C. CAPULA.—5. C. MINUTISSIMA.—6. C. LANGLOISII.—7. C. PORRIGENS —8. C. CARICINA.—9. C. CUPULAEFORMIS.—10 C. TEXENSIS.—11. C. FUMOSA.—12. C. MELLEA.—13. C. VILLOSA.—14. C. RAVENELII.—15. C. CONGLOBATA.—16. C. TILIAE.—17. C. FASCICULATA.

COCKAYNE, BOSTON.

SOME ŒNOTHERAS FROM CHESHIRE AND LANCASHIRE[1]

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Observations

Œnotheras are known to have been naturalized on the Lancashire coast since 1805, and probably existed there much earlier. They are now found on the sand dunes in many places, from Liverpool and the vicinity of Birkenhead northwards along the coast to Southport and Blackpool. They are notably abundant at St. Anne's-on-Sea, where they have been described by Bailey ('07), and in certain localities near Birkenhead (MacDougal '07). I have grown, chiefly at the Missouri Botanical Garden, extensive cultures of plants from the latter region, from seeds obtained through Dr. D. T. MacDougal in 1907, and have visited the Lancashire coast in 1910 and again in July, 1914, when I travelled along the coast from Liverpool to Southport and from Blackpool to St. Anne's. The œnotheras everywhere appear to be spreading, although children gather the flowering shoots in armfuls. The profusion of individuals is greatest at St. Anne's, where acres of waste land in the town are dotted over with them. Smaller colonies occur in various other places, notably at Bidston Junction, near Hightown and at Formby. Small groups of half a dozen plants are sometimes found in isolated places on the dunes.

I will first refer to some of these colonies as I saw them during my last visit, and will then describe a few of the many forms observed in cultures.

The Bidston Junction colony, referred to in MacDougal ('07), is a compact and almost uniform one occurring on a triangular piece of ground between railway tracks, about five minutes' walk down the foot path from Bidston Junction towards Wallersy, on the right-hand side. Some years ago, quantities of sand were dumped here from the coast between Wallersy and New Brighton. Soil from neighboring gardens has also been

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deposited here, and the advent of the œnotheras is doubtless from one or other of these two sources.

The plants closely resemble the "Isle of Wight" race of *Œ. Lamarckiana* (to be described in a book now in process of publication) and the species as it generally appears in English gardens. The rosettes in this colony differ in having green midribs (both dorsally and ventrally), or pink midribs (both dorsally and ventrally), but the depth of red varies. The same applies to the stem-leaves. This is curiously different from other races, such as *Œ.* mut. *rubrinervis*, in which the midribs are red dorsally and green ventrally. The rosette leaves are usually nearly or quite smooth, but some may be crinkled. The plants were short, their average height being about twenty-two inches, though some reached a height of over three feet. The stems bear many red papillæ. The smaller plants were unbranched, the lower stem-leaves being closely crinkled and curled while the upper leaves and bracts are often quite smooth. A peculiarity of the race was the irregular disposition on the stem of much-crinkled and nearly smooth leaves, without gradual transitions between them such as usually occur in de Vries's race of *Œ. Lamarckiana*. Not infrequently crinkled and smooth leaves alternate. The buds have fewer long hairs than in the above mentioned race, and the sepals have uniformly the red color pattern 5–7 of *Œ.* mut. *rubrinervis*, though they vary somewhat in depth of shade. The dimensions of the flowers were as follows: bud cone 50 mm., hypanthium 43 mm., ovary 11 mm., diameter of cone at base 11 mm., length of petals 50 mm., width 60 mm. One plant was identical with the race of de Vries, except in its larger flowers, reddish sepals and fewer long hairs. In most plants there is also a strong distinction between the smooth and crinkled leaves.

This colony differs, therefore, in minor peculiarities from any race of *Œ. Lamarckiana* previously observed, and it exhibits a relatively narrow range of variation.

Along the electric railway tracks north of Liverpool, between Crosby and Hightown, an equally extensive and uniform colony of *Œ. biennis* was found. Thousands of plants, in flower and rosettes, were growing on uncultivated land with a nearly pure sandy soil, behind the coast range of sand hills in a long narrow

area near a clump of small poplar trees. Near the upper end of this area the plants differed in having smaller flowers (petals 21 mm.) and narrow leaves (20 mm. broad). The remainder of the plants had somewhat larger flowers (petals usually 25–27 mm. long), and broader leaves (extreme width 50 mm.).[1] This was almost the only variation observed, and the race comes very close to the type of *Œ. biennis* L. The dimensions of the buds were as follows: bud cone 20 mm., hypanthium 25 mm., ovary 11 mm., anthers surrounding the stigma. The rosette-leaves and stem-leaves *all* have red midribs both dorsally and ventrally. On the same stem some leaves are smooth and some more or less crinkled. The buds are green, devoid of red, with some long hairs, and there are no red papillæ on any part of the plant. Some of the larger plants are well-branched and with very stout stems, a huge pith and a very narrow ring of wood.

This colony is even more uniform than the previous one, and must have originated from one or a very few plants.

Small colonies of *Œ. biennis* were seen at Formby, near the station and in other places. A race of *Œ. Lamarckiana* also grows here on the dunes, although I did not succeed in finding the spot, but local gardens cultivate it. The species is depicted, however, in a rose window erected in St. Luke's Church, Formby, in 1898, containing representative plants of the local flora. The central portion of the window is divided hexagonally and in the six sections the evening primrose alternates with the sea holly. The foliage and large flowers of the former are distinctly shown. Around the margin of the window are *Pyrola rotundifolia* and irises.

At Blundell Sands, near Crosby, a small colony of *Œ. Lamarckiana* was seen on waste ground, and again on the extensive sand dunes between Birkdale and Ainsdale, near Southport. In the latter case there were only three plants, and these possessed red sepals, color pattern 7, green midribs, crinkled leaves, and about $\frac{n}{4}$ long hairs.

By far the greatest abundance of plants was found at St.

[1] These apparently correspond to *Lysimachia virginiana altera, foliis latioribus, floribus luteis majoribus*, Cat. Altdorff. See Gates, R. R. The mutation factor in evolution [pp. 61, 65, 70]. Macmillan. London.

Anne's. In addition to those in the town, which are in great profusion, numerous smaller colonies are scattered along the adjacent sand dunes. The great majority of the plants is the same as at Bidston Junction except in the crinkling of the leaves, having foliage closely resembling that of de Vries's *Œ. Lamarckiana*, midribs red both above and below, the red absent in some individuals. The flower measurements were, length of petals 50 mm., hypanthium 45 mm., ovary 10 mm. Several aberrant individuals were also observed. One dwarf mutant was found growing in the shade of a large plant. It resembled *Œ.* mut. *nanella* but had red midribs. One large rosette, having leaves very obtuse and pale pink midribs, probably belonged to *Œ.* mut. *brevistylis*. A number of plants represented a shorter spindling type with very narrow rosette-leaves (18 mm. wide x 14 cm. long). Another plant belonged to a new type, large and branching with thicker, narrower leaves (33 mm. x 13 cm.), stiffer and narrowly pointed, midribs white, and later in beginning to flower (buds only half developed, July 16).

In addition to these probable mutants, there were found in one field a few plants of a small-flowered *Œ. biennis* race growing with the *Œ. Lamarckiana*. They differed from the latter only in the small flowers (petals 22 mm., style short), and hence were unlike the *Œ. biennis* race previously described. Near by were also found plants, evidently hybrids of these two races, with petals about 30 mm. in length.

CULTURES

Some of my cultures of œnotheras from near Birkenhead have already been described in a general way (Gates, '13). Here I wish to describe a few of these forms in detail, and also to refer to my experiments with plants from St. Anne's. I have not seen the colony from which the Birkenhead seeds were obtained, but it evidently contains a great profusion of forms belonging to both *Œ. Lamarckiana* and *Œ. grandiflora*, while all the colonies I have observed have a much more uniform population.

Œ. MULTIFLORA

One of the distinct races in these cultures I have already (Gates, '10) referred to as *Œ. multiflora*. It is descended

entirely from one individual from a sowing of Birkenhead seeds at Woods Hole in 1908. From this individual an F_1 of 376 plants was grown in the two following years. About 4 per cent of these plants showed virescence, as described in the above paper. In 1910 a total of 297 plants were grown, most of which belonged to the F_2. An F_3 numbering 193 plants in nine families was grown in 1911, and an F_4 of 356 plants in eight families in 1912. The plants were by no means uniform, and they varied considerably from year to year. The description given is therefore a generalized one, and the condition of variability is no doubt similar to that of many wild "species." By isolating the offspring of a larger number of individuals, no doubt this variation could have been further analyzed, but more pressing problems have prevented this being done.

Plate 20 fig. 1 shows a typical rosette of my 1909 culture, pl. 20 fig. 3 the full-grown plant, and pl. 20 fig. 6 a flowering shoot on a larger scale. Specimens of this species are preserved in the herbarium of the Missouri Botanical Garden from my cultures of 1909, and in the British Museum (Natural History) from the 1912 families.

Description: Rosette of few leaves, broad and obtuse-pointed, somewhat crinkled. Full-grown plant pyramidal in outline, with lateral branches and persisting rosette leaves. Average height about 88 cm. Stems slender, stem-leaves smooth, lanceolate, bracts broadly cuneate at base with a very short petiole, tip long-pointed, more or less curled, margin irregularly repand-denticulate. Inflorescence compact, flowers numerous; buds squarish, slender with very long and slender sepal tips, sepals thin, bud cone 35 mm. long, hypanthium 37 mm., sepal tips 7 mm., ovary 10 mm., petals 43 mm., very broad and overlapping when flower is open, long hairs fairly numerous. Few red papillæ on main stem, many on side branches. In 1909 culture the buds were all green, but in 1911 they had the red color pattern of *Œ.* mut. *rubrinervis* and the stems were also reddish.

As regards variations, virescence appeared in the first two generations but not in the last two. On the other hand, a var. *elliptica* was first observed in F_2 and further studied in F_3 and F_4. This variety differs essentially in being smaller and having narrower leaves and narrow, more or less elliptical petals. Plate

20 fig. 2 shows a rosette of this variety in F_3 (1911). One family of 50 plants in 1910 contained 5 of this variety. Usually these plants show partial variability, some flowers having broad petals and others narrow and elliptical ones. Even the different petals of the same flower may show these differences. Flowers with elliptical petals are invariably smaller and are frequently found on the side branches when those of the central stem have normal petals. Hence this variation may be a matter of strength in the plant. The variation, from petals which are broad and truncate or emarginate to those which are narrow and elliptical, or even almost cruciate, is continuous. Thus on one plant in 1911, the dimensions of the petals in two flowers were as follows:

Flower 1. Petal (1) 31 mm. x 21 mm.
Petal (2) 25 mm. x 17 mm.
Petal (3) 20 mm. x 12 mm.
Petal (4) 22 mm. x 13 mm.

In this flower the petals are very small and very unequal in size but all elliptical.

Flower 2. Petal (1) 38 mm. x 39 mm.
Petal (2) 37 mm. x 37 mm.
Petal (3) 34 mm. x 36 mm.
Petal (4) 35 mm. x 36 mm.

In this flower the petals were nearly full size, nearly equal, and scarcely elliptical.

The inheritance of this condition is on a sliding scale, plants with only broad petals giving some offspring with elliptical petals, and plants with elliptical petals giving some offspring having only broad petals, though in the latter case the plants bearing elliptical petals are more numerous than in the former case. Thus the F_3 family from a normal plant contained 14 specimens having broad petals only and 15 having some elliptical petals; while another F_3 family of 44 plants derived from a plant having elliptical petals contained only 5 plants having exclusively broad petals. These peculiarities of the petals are probably to a large extent under the control of environmental features such as temperature and water supply.

The difference between broad and narrow leaves is much sharper. Thus in my F_4 cultures in 1912 certain families contain

both the broad or normal type (pl. 20 fig. 5) and the *elliptica* variety (pl. 20 fig. 4). The latter had a number of flowers with elliptical petals and it also had a different method of branching. Plate 21 fig. 12 is representative of a uniform F_4 culture of 49 plants of the variety *elliptica*. This photograph is taken on a larger scale, and the nodding of the stem is merely due to wilting. This differs from *typica* (pl. 20 fig. 5) constantly in having narrower leaves and short branches, as well as in the occasional elliptical flowers which appear to be largely under environmental control.

The variability of this race is therefore as interesting as are the features, such as the general bud and leaf characters, in which it is constant. The fact should also be mentioned that a *lata*-like mutant, doubtless having 15 chromosomes, appeared in the F_4 generation, and also a mutant resembling *Œ.* mut. *albida*.

Œ. RUBRINERVOIDES

This race resembles *Œ.* mut. *rubrinervis* in many features, and yet differs from it constantly throughout. I have previously referred to this Birkenhead race as No. 25 (Gates, '11, p. 350) and studied the variation of the red stripes on the buds. In all, 1968 plants of this race have been grown in the years 1909–1912, so that four generations of offspring from a single individual have been cultivated. An illustration of that individual has already been published (Gates, '12, pl. 3). One family of offspring was grown in 1909, two in 1910, eight in 1911 and nine in 1912. Usually the variability of families progressively decreased, since each family was derived from the selfing of one individual of the previous generation. The discussion of the precise ancestry of this race is of course out of the question, but its characters bear nearly though not quite the same relation to the *Œ. Lamarckiana* from this region that the *Lamarckiana* and *rubrinervis* of de Vries's cultures bear to each other.

The 1909 family, or F_1, numbered 111 plants. Plate 21 fig. 8 shows one of these as a rosette. The leaves are narrower and more pointed than in mut. *rubrinervis*, and nearly smooth. About 20 of the plants in this culture omitted the rosette stage altogether and shot up a stem directly from the seedling stage (pl. 20 fig. 7). A normal mature plant of this family is shown

in pl. 21 fig. 11. It will be seen that there is no indication of a rosette, and the branching is quite different from that of *Œ.* mut. *rubrinervis*. In many cases, however, a rosette is formed. When the rosette is omitted the branching is changed. Plate 21 fig. 10 shows on a larger scale another individual in flower. The stem-leaves differ from those of *Œ.* mut. *rubrinervis* in being narrower, more pointed and smoother.

In this race the red papillæ on the stem were very numerous, and the buds likewise were slightly more red than in *Œ.* mut. *rubrinervis*. The modal color pattern of the whole population was *5* as in *Œ.* mut. *rubrinervis*, but plants with their mode at *7* were much more numerous than in the latter (see Gates, '11, p. 351). The race as a whole inherited the capacity for producing a slightly greater amount of pigment. The ovary usually bore many long hairs arising from red papillæ; on the hypanthium were few long hairs from slight green mounds; and on the bud cone scattered long hairs from conspicuous red papillæ. In occasional buds, when the color pattern was only *3*, the green papillæ were more numerous. In addition to the color pattern of the sepals there was usually weak red on the hypanthium.[1]

The same conditions as regards pigmentation have been maintained in later generations. The plants were, however, by no means uniform in all respects, and this was not to be expected since they were derived from one individual of a freely intercrossing population. Plate 21 fig. 9 represents a rosette of one of the F_2 plants. The latter differs obviously from the one represented in pl. 21 fig. 8, but the race retained in this and subsequent generations the long, narrow, smoothish leaves as well as the pigmentation. The various F_3 and F_4 families, each derived from a selfed individual, produced sub-races differing more of less from each other and varying within narrower limits. It does not appear that the Mendelian theory of the sorting out of factors, or "genes," affords an adequate explanation of all these phenomena.

[1] Since this condition of bud-pigmentation resembles that obtained in certain F_2 and F_3 hybrids of *Œ.* mut. *rubricalyx* and *Œ. grandiflora* (see Gates '14), it is possible that it may have arisen in a similar way, i. e., by the appearance of a red-budded mutation which subsequently crossed with other species, in which crosses some blending of pigmentation occurred giving rise to the present condition.

Œ. TARDIFLORA

This name I have used for another race having many peculiarities and showing more resemblance to *Œ. grandiflora* in its flowers and foliage. It is race No. 52 from the same source as the above. A single individual produced in 1909 nineteen plants which were fairly uniform. The rosettes contained only a few leaves, but large plants were formed, one of which is shown in pl. 22 fig. 17. Although this photograph was taken on August 21, the plants with one exception had not begun to flower. The leaves resembled those of *Œ. grandiflora.* They were large with long and acute tips, tapering to the bases, often bearing reddish blotches, sometimes much curled, somewhat crinkled along the midrib. The margin was conspicuously serrately toothed (see pl. 22 fig. 17). At the end of the season (September) these plants came into bloom, and pl. 22 fig. 20 shows a plant photographed on October 2. The buds resembled those of *Œ. grandiflora* but were small. The bud cones were pointed, smooth and rounded, the petals slightly larger than in *Œ. biennis*, or in a few cases much larger. The petals were also deeply emarginate, strongly cuneate and narrow; and the bracts were very small, narrowly lanceolate and yellowish, giving a peculiar appearance to the flowering shoot. The margins of the bracts were nearly entire or in some cases distantly denticulate.

The offspring of the plant in pl. 22 fig. 20 were grown and showed the same peculiarities. The race has not been cultivated further. It was doubtless of hybrid origin and was more nearly allied to *Œ. grandiflora* than to the *Lamarckiana* complex.

Œ. RUBRITINCTA

Reference may be made to one further race which was known as "type M." It originated from one plant in a sowing of the Birkenhead seeds in 1909. It will be understood that scarcely two plants from this sowing were alike, but some were much more distinct than others. The plant in question was a handsome one with very narrow leaves and bright red midribs. Its offspring, grown in 1911, were lost with the exception of one plant which was the same as the parent. It is shown in pl. 22 fig. 16. The basal leaves were very long with long petioles, the stem leaves very narrow, smooth, with margin closely repand-

denticulate, blade narrowing gradually to a very short petiole, midribs and petioles bright red dorsally and ventrally; lowermost bracts 17 mm. in width by 9 cm. in length, upper bracts 11 mm. wide by 58 mm. in length. The buds most resemble those of *Œ. grandiflora*, being nearly devoid of long hairs, slender and somewhat rounded, with setaceous sepal tips and some red on the sepals; length of petals 32 mm., hypanthium 43 mm., sepal tips 9 mm., ovary 10 mm.

In 1912 three families of F_2 offspring, numbering in all 236 plants, were grown from the plant just described. All three families agreed in containing several types exhibiting a remarkable degree of variability.

An attempt was made to place the plants in five classes, but the categories overlapped and made classification for the most part impossible. The majority of the plants resembled the parent individual in their main features but they varied enormously in width of leaf from broad (21 mm.) to very narrow (8–6.5 mm.). These conditions were connected by intermediates, and, moreover, there were considerable variations within the individual, one branch with very narrow leaves being found on a plant with broad leaves. In addition to these variants, the three families contained 35 dwarfs, or 14.8 per cent, and the latter varied in leaf-width in the same remarkable manner. The dwarfs agreed only in having short internodes. Two of them are shown in pl. 21 figs. 13, 14, the former having narrow leaves and extremely short internodes, the leaves of the latter being quite linear. The plant would never be taken for an œnothera.

The advent of a large percentage of dwarfs in this family is similar to their occurrence in other *Œ. grandiflora* races from that locality (see Gates, '14, p. 246). The precise manner in which this capacity for producing dwarfs is inherited, is a difficult question which need not be considered here, particularly as it has been discussed elsewhere (Gates, '14).

Plate 22 fig. 15 represents one of the *Lamarckiana*-like rosettes from this source, grown in 1909. Others approached de Vries's race more closely, to the point of identity. Plate 22 figs. 18, 19 represent selected rosette-leaves taken from this culture to show the range of types exhibited. Such leaves as the

two on the right in pl. 22 fig. 18 were greatly overgrown and were far larger than ever appear even in *Œ.* mut. *gigas*. These forms have not been sufficiently studied since to give an adequate account of them.

It will be obvious that the forms described here under the names *multiflora, multiflora elliptica, rubrinervoides, tardiflora* and *rubritincta* are not pure species or even true-breeding races. They are undoubtedly as diverse from each other as average species, however, and many systematic species if bred experimentally would probably not breed true within narrower limits than these races have done. One feature of interest attaching to these races is the fact that the main type persists essentially unchanged, though various mutants and heterozygous forms are thrown off. The behavior is not, in the main, like the Mendelian process of recombination. Repeated selfing of each race usually decreases its variability by eliminating various hybrid elements. But this process does not extend to the basal differences between the races, which, as we have seen, remain as unlike as they were before. In this aspect the hereditary behavior of these races resembles that of *Œ. Lamarckiana*. But there are a number of differences which I need not fully consider. Thus *Œ. multiflora* gives rise to its variety *elliptica* much as though it were split off from a heterozygous condition, and the variability of *rubritincta* in leaf-width, as well as its production of numerous dwarfs, is unlike anything in the behavior of *Œ. Lamarckiana*.

Many other equally distinct types were derived from this locality (see, e. g., pl. 22 figs. 18, 19), but they have not been cultivated in subsequent generations.

Œ. LAMARCKIANA FROM ST. ANNE'S

In 1910 I obtained seeds from a colony of *Œ. Lamarckiana* growing by the Manchester Children's Hospital Convalescent Home, at St. Anne's-on-Sea. Many of these were found in later cultures to agree exactly with the *Lamarckiana* of de Vries except in the red color pattern of the sepals. I was formerly inclined to lay little stress on this difference but there is no doubt that it is inherited. The fact therefore remains that a precise duplicate for de Vries's race of *Œ. Lamarckiana* is relatively

infrequent on the Lancashire coast, although many forms approach it very closely and differ only in this one feature. As will be seen below, certain other plants agreed with de Vries's *Lamarckiana* except in the shape of the buds.

In 1911 a sowing of the seeds yielded 22 plants. The rosettes were for the most part uniform and very similar to *Œ. Lamarckiana,* two, however, having red midribs and lighter green leaves (*rubrinervis* type). One plant was aberrant, resembling *Œ.* mut. *semilata* in its buds, which were, however, small as in *Œ. biennis.* The bud cone was also somewhat rounded and barrel-shaped, length of ovary 11 mm., hypanthium 37 mm., cone 19 mm., petals 22 mm., style short so that anthers surround base of stigma. The features of this plant make it scarcely likely that it arose as a hybrid. It produced plenty of pollen and seeds.

Another sowing of these seeds in 1912 yielded 140 plants, which included one mut. *lata* with bad pollen (doubtless having 15 chromosomes) and one variegated *Lamarckiana* plant. The variegation was noticed when the plant was a young seedling. It reached maturity and proved to be a periclinal chimæra. Nearly all the leaves were variegated green and yellow. Many leaves were green bordered with yellow, showing the absence of chloroplasts from the epidermal and probably also the hypodermal layer. Occasional leaves were almost entirely yellow, and some were yellow on one side of the midrib and green on the other. There were also broad white bands on the margin of the sepals. The pollen was abundant and plenty of seeds were set.

Two sowings of seeds from this plant were made in 1912. The seeds numbered respectively 121 and 145. Only two seeds in one pan were observed to germinate, and the seedlings quickly died, probably from lack of chlorophyll. Regarding the origin of this periclinal mutation, it would appear to have originated in the embryo after fertilization through the loss of chloroplasts from the outer layers of the growing point.

The foliage in the rest of the culture agreed with the type of *Œ. Lamarckiana.* One plant differed in having stem-leaves more or less pointed at the base, not crinkled, midribs pink, and smaller flowers (petals 29 mm. long x 38 mm. broad, style short, buds

squarish). Two other plants agreed exactly with *Œ. Lamarckiana* except in the buds. The petals were 35 mm. long x 48 mm. broad, emarginate, anthers reaching nearly to top of stigma lobes, sepals green and with the same pubescence as in *Œ. Lamarckiana*, from which these two plants therefore differed only in the somewhat smaller flowers and shorter style. One mut. *nanella* also occurred in this culture, and several other slightly aberrant individuals, including a plant with broadly elliptical foliage. The "*Lamarckiana* foliage" was also more variable than in cultures from de Vries, this no doubt being due to the continued inbreeding in the latter case.

It will be understood that the new forms described here are scarcely to be looked upon as "new species" according to the usual interpretation at the present time. They merely represent a partial analysis of a complex interbreeding colony of forms, and their variability is one of their most interesting features. Nearly all if not all the differences observed are inherited, however, and the mutations can in many instances be separated from the characters arising through hybridization. The forms are, moreover, as distinct from each other as many species of *Œnothera*.

In conclusion, I am indebted to the Missouri Botanical Garden and the John Innes Horticultural Institution for the facilities provided for growing the plants, and to Mr. E. J. Allard for several of the photographs. A portion of the expenses of my second visit to Lancashire was defrayed by a grant from the Royal Society.

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Explanation of Plate

PLATE 20

Fig. 1. *Œ. multiflora*, rosette, 1909.
Fig. 2. *Œ. multiflora elliptica*, rosette, 1911.
Fig. 3. *Œ. multiflora*, full-grown plant, 1909.
Fig. 4. *Œ. multiflora elliptica*, 1912.
Fig. 5. *Œ. multiflora*, 1912.
Fig. 6. *Œ. multiflora*, flowering shoot, 1909.
Fig. 7. *Œ. rubrinervoides*, young plantlet showing absence of rosette, 1909.

GATES—OENOTHERAS

COCKAYNE, BOSTON.

Explanation of Plate

PLATE 21

Fig. 8. *Œ. rubrinervoides*, rosette, 1909.
Fig. 9. *Œ. rubrinervoides*, rosette, 1910.
Fig. 10. *Œ. rubrinervoides*, showing nearly smooth, pointed leaves, 1909.
Fig. 11. *Œ. rubrinervoides*, no rosette, 1909.
Fig. 12. *Œ. multiflora elliptica*, 1912. (Tip of plant drooped from wilting.)
Fig. 13. Linear-leaved dwarf in offspring of *Œ. rubritincta*, 1912.
Fig. 14. Dwarf offspring of *Œ. rubritincta*, 1912.

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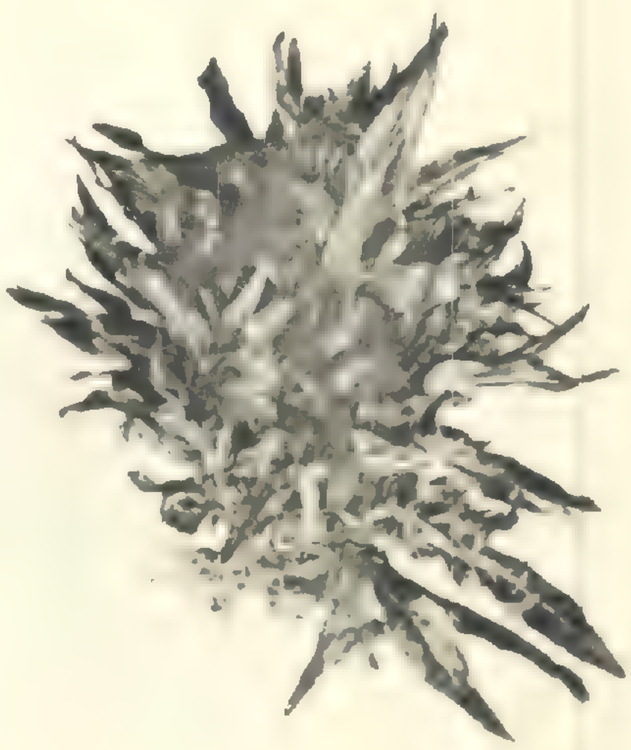

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GATES—OENOTHERAS

Explanation of Plate

PLATE 22

Fig. 15. *Œ. Lamarckiana*-like rosette, 1909.
Fig. 16. *Œ. rubritincta*, 1911.
Fig. 17. *Œ. tardiflora*, showing serrated leaves and absence of flowers, August 21, 1909.
Fig. 18. Selected leaves from various rosettes, 1909.
Fig. 19. Selected leaves from various rosettes, 1909.
Fig. 20. *Œ. tardiflora*, showing late appearance of buds, October 2, 1909.

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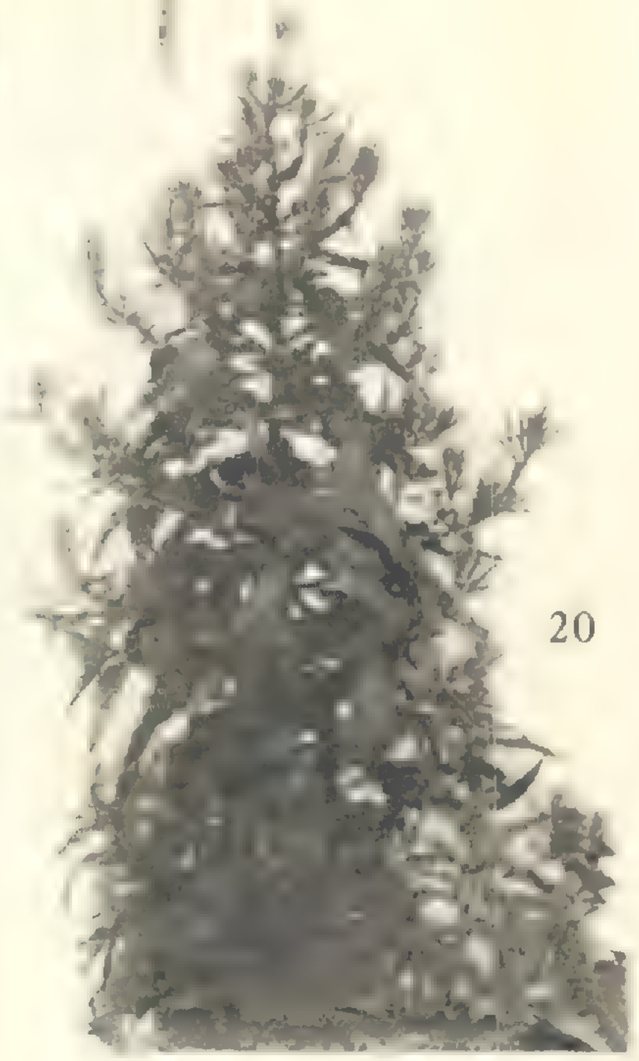

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GATES—OENOTHERAS

COCKAYNE, BOSTON.

A TEXAN SPECIES OF MEGAPTERIUM[1]

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While looking over some material in the herbarium of the Missouri Botanical Garden, a sheet was found containing three specimens which were so distinctive that it seemed desirable to describe them. The interest in them was enhanced by the fact that one of the specimens differs strikingly from the other two in such a way as to suggest that it may be a mutation. The plants in question were collected at Amarillo Creek, in Northern Texas, by J. Reverchon, who had recognized them as representing a new species of *Megapterium*.

I am indebted to Dr. Greenman for suggesting a very appropriate name for this species. The diagnosis is as follows:

Megapterium argyrophyllum, sp. nov. Plate 23. figs. 1 and 2.

Herba cæspitosa; foliis lanceolatis, petiolatis obscurē glanduloso-denticulatis, utrinque densē canescento-pubescentibus; caulibus et alabastris (hypanthio et ovario incluso) canescente pubescentibus; ovarium quadrialatum, pedicellatum; hypanthium 9–10 cm. longum, paulatim ad basin coni dilatum; petala 3–4 cm. longa.

Var. **retusifolium, var. nov.** Plate 23. fig. 3.

A forma typica differt foliis subrotundis bis oblongo-obovatis, retusis, mucronatis; floræ grandioræ (petala 45 mm. longa).

Specimens examined:

Texas: on rocky bluffs at Amarillo Creek, in northern Texas, 29 May, 1902, *J. Reverchon, 2749* (Mo. Bot. Gard. Herb.), TYPE; stony bluffs along Red River, Randall Co., northern Texas, 12 August, 1900, *H. Eggert* (Mo. Bot. Gard. Herb., 4 sheets).

Two of the specimens, one slightly older than the other (see pl. 23 fig. 1, 2), represent the type of the species. The plants are cæspitose or with very short internodes, leaves coriaceous, lanceolate, broad-pointed, tapering below to a petiole, about

[1] Issued January 30, 1915.

8 cm. long by 2 cm. in greatest width, margin distantly and obscurely glandular-denticulate, very densely and uniformly covered on both surfaces with an appressed canescent pubescence of long, pointed, tuberculate hairs. Stems and buds less densely covered with the same type of pubescence, ovary four-winged, 10–15 mm. in length, densely canescently pubescent, pedicellate; hypanthium 9–10 cm. in length, 2–2.5 mm. thick, gradually widening to base of cone; bud cone 30–35 mm. in length, diameter at base 8 mm., sepal tips appressed, 3–4 mm. in length, petals 3–5 cm. long, stigma surrounded by or slightly exceeding the stamens; capsules immature.

The remarkable canescent pubescence covering the whole plant, as well as the cæspitose habit, distinguish this species from *Megapterium missouriensis* (Sims) Spach, and *M. macrocarpum.*[1] The flowers are also smaller, there are no purple spots on the sepals, and the hypanthium is shorter than in these species, which differ in foliage as well. The present species is apparently perennial. Its nearest relative is *M. Fremontii* (Watson) Britton, from which it differs in the more cæspitose habit, larger flowers, and much broader leaves.

The variety *retusifolium* is founded on the third specimen on the sheet (see pl. 23 fig. 3). It differs sharply from the species in the shape of the leaves, which are very broad and blunt at the point, subrotund to oblong-obovate, retuse, and distinctly mucronate. The margin of the leaves is also nearly or quite entire. The flowers are larger (petals 45 mm., bud cone 9 mm. in diameter at base). Microscopic examination of the hairs disclosed considerable variation in size, but apparently no constant difference from those of the species.

The Eggert specimens, while obviously belonging to the same species, show much more variability in foliage. The leaves vary on different specimens from narrowly lanceolate (9 mm. in width) to broad oblong-lanceolate (30–36 mm. wide) and acuminate. The latter resemble var. *retusifolium* except the leaf tips, which are only slightly retuse in one specimen. One of the broad-leaved specimens also has a smaller flower (petals 20 mm.). Cultures from seeds from this locality would doubt-

[1] **Megapterium macrocarpum** (Pursh), comb. nov.
Œnothera macrocarpa Pursh, Fl. Am., Sept. 2: 734. 1814.

less disclose a considerable number of forms. The ripe fruits from these specimens are broadly winged, nearly orbicular, about 35 mm. long and 25 mm. wide, retuse or acuminate at the apex.

Examination of herbarium specimens of *M. missouriensis* (Sims) Spach makes it evident that the polymorphism in this species as now understood is quite as great as in many species of *Œnothera*. There are included races varying in amount and character of pubescence, in width of leaf from broadly lanceolate to almost linear, in presence or absence of purple spots on the sepals, in size of flower, and other features.

Explanation of Plate

PLATE 23

Figs. 1 and 2. *Megapterium argyrophyllum.* From the type specimens, J. Reverchon, No. 2749 in part, in the Herbarium of the Missouri Botanical Garden.

Fig. 3. *M. argyrophyllum* var. *retusifolium.* From the type specimen, J. Reverchon No. 2749 in part, in the Herbarium of the Missouri Botanical Garden.

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GATES—MEGAPTERIUM

COCKAYNE, BOSTON.

DIAGNOSES OF FLOWERING PLANTS, CHIEFLY FROM THE SOUTHWESTERN UNITED STATES AND MEXICO[1]

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The present paper is the result of a study of several collections of plants from the southwestern United States and Mexico, especially the relatively large series of specimens secured by Mr. Harley P. Chandler at Rio Hondo, Texas, and by Mr. Charles Russell Orcutt along the Texas-Mexican boundary and in various parts of Mexico. These collections have been received at the Missouri Botanical Garden for identification and incidental to the work thereto the following plants seem to the writers to be worthy of record and characterization.

Anthericum (Hesperanthes) Chandleri Greenman & Thompson, sp. nov.

Fibræ radicales carnosæ apice clavatæ, collo radicis parce fibroso; foliis plurimis 12–15 graminoideis planis lanceolato-linearibus sensim attenuatis acutis 3.5–4.5 dm. longis 7–10 mm. latis circiter 24-nerviis cum venis transversis conjunctis utrinque glabris integerrimis; scapo 1 m. alto tereti glabro bracteato, bracteis plus minusve foliiformibus sursum gradatim reductis; inflorescentiis paniculatis usque ad 3.5 dm. longis glabris, racemo terminali 2–2.5 dm. longo, racemis lateralibus 1–1.5 dm. longis, bracteis triangulari-acuminatis vel lanceolato-attenuatis subscariosis 3–20 mm. longis; floribus 2–4 in axillis bractearum; pedicellis 10–12 mm. longis infra medium articulatis; perianthio pallido-flavo vel stramineo, laciniis oblongo-lanceolatis triner-viis circiter 1 cm. longis; staminibus perianthio duplo brevioribus, filamentis muricatis; stylo 8 mm. longo glabro; capsula matura ignota.

[1] Issued January 30, 1915.

Specimen examined:

Texas: vicinity of Rio Hondo, Cameron County, September, 1913, *Harley P. Chandler, 7059* (Mo. Bot. Gard. Herb.), TYPE.

This species belongs to the subgenus *Hesperanthes* according to Baker's treatment of this group (Jour. Linn. Soc. Bot. **15**: 253–363. 1876); it is apparently most nearly related to *A. stenocarpum* Baker, a co-type of which is in the herbarium of the Missouri Botanical Garden, from which it is readily distinguished by the broader leaves, entire leaf-margins, the presence of anastomosing cross-veins, and by the leafy scape and yellow flowers.

Zephyranthes chrysantha Greenman & Thompson, sp. nov.

Bulbus subglobosus 2–2.5 cm. diametro tunicis brunneo-nigrescentibus vestitus, collo 3–5 cm. longo 6–8 mm. diametro; foliis 2–4 sub anthesi evolutis linearibus 2.5–4.5 dm. longis 2–3 mm. latis glabris; scapis 2–3 dm. altis glabris; spatha membranacea 2.5–3.5 cm. longa inferne tubulosa, tubo 1–1.5 cm. longo, lobo unilaterali lanceolato 1.5–2 cm. longo; pedicellis 2.5–3.5 cm. longis gracilibus; perianthio infundibuliformi 3–3.5 cm. longo flavo 6-lobato, tubo cylindraceo circiter 5 mm. longo, lobis oblanceolatis 3–3.2 cm. longis 5–12 mm. latis acutis staminibus ad apices tubi perianthii insertis segmentis perianthii duplo brevioribus; stylo brevitrilobato staminibus subæquantibus; capsula depresso-globosa 10–12 mm. longitudine et diametro, seminibus numerosis irregulariter compressis 5–6 mm. longis 2–5 mm. latis atratis et sæpe nitidis.

Specimen examined:

Texas: Rio Hondo, Cameron County, September, 1913, *Harley P. Chandler, 7056* (Mo. Bot. Gard. Herb.), TYPE.

The species here characterized is allied to *Z. Eggersiana* Urb., particularly in the size and color of the flowers, but differs in having more numerous and broader leaves, shorter perianth-tube and longer spathes.

Sisyrinchium angustissimum (Rob. & Greenm.) Greenman & Thompson, comb. nov. Plate 24.

S. alatum Hook. var.? *angustissimum* Rob. & Greenm. Am. Jour. Sci. **50**: 166. 1895.

Radices carnoso-fibrosi fasciculati; caulibus erectis strictis vel

subflexuosis 2.5–9 dm. altis multo-ramosis angustissime ancipiti-alatis foliosis glabris vel obscure hirtello-puberulentis basi reliquiis brunneis fibrosis squamarum et foliorum primorum obtecto; foliis radicalibus linearibus gramineis usque ad 4.5 dm. longis 1–4 (rarius 6) mm. latis crebrenerviis glabris vel marginibus hirtellis, eis caulinis conformibus sed sursum gradatim reductis; spatha diphylla, bracteis foliiformibus 1.5–2 cm. longis glabris marginibus plus minusve purpurascentibus, pedicellis 2–4 ex eadem spatha 1.5–2.7 cm. longis gracilibus glabris; perianthio profunde 6-partito verisimiliter flavo, lobis ovato-ellipticis acutis vel emarginatis et submucronatis 5–7-nerviis; ovario oblongo-obovato juventate sæpe pubescenti glabrato; capsula matura oblonga 5–10 mm. longa 4–6 mm. diametro glabra, seminibus subglobosis circiter 1.5 mm. diametro in sicco nigrescenti.

Specimens examined:

Mexico: State of Oaxaca, Sierra de San Felipe, altitude 2895 m., 22 June and 29 August, 1894, *C. G. Pringle, 4703* (Mo. Bot. Gard. Herb.), CO-TYPE; Sierra de San Felipe, altitude 3048 m., August–September, 1894, *Charles L. Smith, 758* (Mo. Bot. Gard. Herb.). State of Morelos, lava beds above Cuernavaca, altitude 2590 m., 19 November, 1902, *C. G. Pringle, 11191* (Mo. Bot. Gard. Herb.). State of Puebla, vicinity of San Luis Tultitlanapa, near Oaxaca, June, 1908, *C. A. Purpus, 3356, 3357* (Mo. Bot. Gard. Herb.).

After a careful reëxamination of the original material on which this variety was based, particularly in the light of additional specimens from subsequent collections, it seems undesirable to retain the plant as a variety of *S. alatum* Hook. Mr. Hooker's species was founded on specimens collected in Demerara, British Guiana, by Dr. Hancock; and specimens secured by Mr. Gardner in the Organ Mountains of Brazil and by Tweedie on the marshes of the La Plata River were considered conspecific. While the writers have not seen any of these specimens, yet from the original description and the illustration accompanying it that species is interpreted as having a broadly winged stem, short and relatively broad ensiform leaves, and broad spathes. These characters can not be applied properly

to the Mexican plant in question. It seems advisable, therefore, to regard the south Mexican plant as a distinct species which may be further characterized as above.

OECOPETALUM Greenman & Thompson, gen. nov. *Icacinaceæ*

Calyx 5-lobus. Petala 5 hypogyna valvata intus costata, margine et apice inflexa. Stamina 5 hypogyna petalis alterna et iis basi cohærentia, filamentis dilatis glabris apice contractis; antheræ erectæ lanceolatæ basi sagittatæ connectivo latiusculo; thecæ lateralæ remotæ et in cavitatibus petalorum receptæ. Discus obsoletus. Ovarium uniloculare, stylus erectus conicus, stigma terminale. Ovulum 1 pendulum. Fructus ignotus.—Frutices vel arbores. Folia alterna coriacea integerrima. Flores cymis brevibus axillaribus dispositi.

O. mexicanum Greenman & Thompson, sp. nov. Plate 25.

Frutex (?) vel arbor (?); ramis cortice griseo tectis; ramulis juventate sericeo-pubescentibus mox glabratis; foliis alternis petiolatis elliptico-lanceolatis 1–2.5 dm. longis 3.5–10 cm. latis brevi-acuminatis obtusis integerrimis utrinque glabris vel praesertim in nerviis sparsissime adpresso-puberulentis subtus palli-dioribus basi sensim angustatis acutis, petiolis 7–15 mm. longis supra canalyculatis; inflorescentiis in axillariis superioribus cymosis plus minusve adpresso-sordido-pubescentibus, pedunculo usque ad 2 cm. longo; floribus cum pedicello articulatis et caducis; calyce griseo-tomentoso parvo circiter 2 mm. alto 5-lobato, lobis ovatis obtusis 1 mm. longis; petalo 5 oblongo-lanceolato 8 mm. longo 2 mm. lato verisimiliter albo utrinque glabro intus longitudinaliter insigniter unicostato; ovario et stylo glabro; fructu et seminibus ignotis.

Specimen examined:

Mexico: State of Vera Cruz, Sierra Madre near Miscantla, August, 1912, *C. A. Purpus, 6159* (Mo. Bot. Gard. Herb.) TYPE.

Specimens of the plant here described were submitted to the Missouri Botanical Garden for determination by Mr. T. S. Brandegee who suggested its probable relationship with *Mappia*. After a careful study of the material at hand it seems unmistakably to belong to the *Icacinaceae*, but until the fruit is known its exact position in the family must remain doubtful.

In habit and in the structure of the flower it possesses certain characters in common with *Mappia, Kummeria* and *Poraqueiba,* but in a combination of the floral characters, particularly in the free or merely coherent glabrous and strongly ribbed petals, the broad smooth filaments, elongated anthers, which in cross section are distinctly x-shaped, and in the single suspended ovule the plant in question differs from the genera above mentioned. Generic rank is therefore given to it and we propose the name **Oecopetalum,** from *οἶκος* house and *πέταλον* petal, in reference to the little recesses or pockets formed by the adjacent petals in which the anthers rest.

Stemodia linearifolia (Morong) Greenman & Thompson, comb. nov.

Stemodiacra linearifolia Morong, Ann. N. Y. Acad. Sci. **7**: 183. 1893.

Stemodia tomentosa (Mill.) Greenman & Thompson, comb. nov.

Erinus tomentosus Mill. Dict. 1768. [8th ed.]—*Herpestes tomentosa* Schlecht. & Cham. Linnæa **5**: 106. 1830.—*Stemodia lanata* Ruiz & Pav. in DC. Prodr. **10**: 383. 1846; Hemsl. Biol. Cent.-Am. Bot. **2**: 450. 1882.—*Stemodiacra tomentosa* O. Kuntze, Rev. Gen. **2**: 466. 1891.

Siphonoglossa Greggii Greenman & Thompson, sp. nov.

Suffruticosa; caulibus erectis vel adscendentibus 0.5–2 dm. longis subcylindratis et sæpe quadrisulcatis pubescentibus in lineis decussatis cum pilis reflexis; foliis oppositis brevipetiolatis lanceolatis vel obovatis 0.5–2.5 cm. longis 3–7 mm. latis acutis vel obtusis vel rotundatis integris basi in petiolum gradatim angustatis supra glabris subtus paulo pallidioribus juventate secundum nervos venasque adpresso-puberulentis; floribus in axillis supernis solitariis sessilibusque, bracteis subspathulatis; calyce profunde 5-partito 4 mm. longo, laciniis lineari-lanceolatis glabris; corolla 1.5–2 cm. longa bilabiata, labio anteriore horizontaliter patenti trilobulato, labio posteriore suberecto emarginato, tubo gracili 9–14 mm. longo extus pubescenti; ovario et stylo glabro; capsula circiter 7 mm. longa glabra, seminibus suborbicularibus compressis verrucosis circiter 2 mm. diametro.

Specimens examined:

Mexico: State of Tamaulipas, Matamoras, 7 June, 1847, *Dr. J.*

Gregg, 915 (Mo. Bot. Gard. Herb.), TYPE; Cervallo, 29 May, 1847, *Dr. J. Gregg, 845* (Mo. Bot. Gard. Herb.).

Texas: Rio Hondo, Cameron County, September, 1913, *Harley P. Chandler, 7081* (Mo. Bot. Gard. Herb.).

The species here proposed is nearly related to *S. Pilosella* Torr. from which it is distinguished by the pubescence of the stem, namely reflexed hairs disposed in decussating lines, somewhat narrower leaves, and uniformly shorter fruit.

Siphonoglossa Pilosella Torr. Bot. Mex. Bound. 124. 1859.

This species is well represented in the herbarium of the Missouri Botanical Garden by a suite of more than thirty specimens. To it should be referred one of Lindheimer's Texas plants, namely number 1065, collected in 1851, which by clerical error was distributed as "*Ruellia Parryi* Gray."

Randia Gaumeri Greenman & Thompson, sp. nov.

Frutex ramosus; caule ramisque cortice griseo glabro tectis; spinis axillaribus usque ad 1.5 cm. longis divaricatis; foliis obovatis 0.5–1.5 cm. longis apice plerumque rotundatis integris basi in petiolum marginatum contractis utrinque glabris vel supra in nervis ad basin puberulentis; floribus axillaribus sessilibus; calyce toto 1–1.5 mm. longo 4-lobato glabro; lobis triangularibus acutis ciliatis; corolla hypocraterimorpha parva 4-lobata, tubo cylindraceo circiter 2.5 mm. longo extrinsecus glabro, lobis contortis ovatis tubo subaequantibus; antheris ad faucem corollæ sessilibus exsertis; ovario biloculari; bacca ignota.

Specimen examined:

Mexico: State of Yucatan, at Izamal, coll. of 1895, *Dr. Geo. F. Gaumer, 589* (Mo. Bot. Gard. Herb.), TYPE.

The divaricately spreading axillary spines, relatively small obovate leaves, and the minute flowers amply distinguish this species from all others of the genus. It is with pleasure that the authors dedicate this new species to Dr. Gaumer, who has done so much to further our knowledge of the flora of Yucatan.

Randia Purpusii Greenman & Thompson, sp. nov.

Verisimiliter frutex; ramis ramulisque cortice brunneo vel griseo tectis; spinis ad apices ramorum plerumque quaternis vel binis, vel rarius nullis, 3–6 mm. longis; foliis lanceolatis vel obovato-lanceolatis 1.5–5.5 cm. longis 0.8–2 cm. latis obtusis

vel acutis integris basi in petiolum marginatum gradatim angustatis supra hirsutis subtus paulo pallidioribus et subtomentosis; stipulis triangulari-ovatis utrinque pubescentibus; floribus sessilibus axillaribus terminalibus; calyce toto 6–7 mm. longo 4-lobato, tubo 1.5 mm. longo sericeo, lobis linearibus vel anguste spathulatis 3–3.5 mm. longis patentibus parce pubescentibus; corolla hypocraterimorpha profunde 4-lobata, tubo cylindraceo fere 1.5 cm. longo extus parce piloso, lobis oblongo-lanceolatis tubo subæquantibus; antheris ad faucem corollæ paulum exsertis; ovario biloculari, ovulis plurimis; fructu ignoto.

Specimen examined:

Mexico: State of San Luis Potosi, Minas de San Rafael, May, 1911, *C. A. Purpus, 5208* (Mo. Bot. Gard. Herb.), TYPE.

Randia truncata Greenman & Thompson, sp. nov. Plate 26.

Frutex erectus 3–4 m. altus ramosus; caule ramisque tereti cortice griseo tectis juventate parce strigulosis mox glabratis; spinis 0.5–1 cm. longis binis ad apices ramorum; foliis obovatis vel spathulatis 0.5–3 cm. longis 0.3–1.7 cm. latis ad apicem rotundatis obtusis vel submucronato-acutis integris utrinque glabris basi in petiolum marginatum plus minusve abrupte contractis; floribus sessilibus axillaribus terminalibus; calyce toto 1.5–2 mm. longo, limbo cupuliformi truncato; corolla hypocraterimorpha in sicco atrato, tubo cylindraceo 1–1.5 cm. longo extus glabro intus sparse piloso, lobis subovatis 4–5 mm. longis 3–4 mm. latis apice rotundato vel brevissime acuminato; antheris ad faucem corollae sessilibus semiinclusis; bacca immatura globulosa circiter 0.5 cm. diametro.

Specimens examined:

Mexico: State of Yucatan, vicinity of Izamal, coll. of 1895, *Dr. Geo. F. Gaumer, 713,* TYPE, and *506* (both in Mo. Bot. Gard. Herb.); road to Progresso north of Merida, 7 April, 1865, *Schott, 262* (Mo. Bot. Gard. Herb.), distributed as "*R. aculeata.*"

Co-types of the above species may be looked for in herbaria under *R. xalapensis* under which name Dr. Gaumer's material cited above was distributed. From this species, however, *R. truncata* differs in the more obovate outline and the less conspicuous veins of the leaf, the somewhat longer and more slender corolla-tube, and in the smaller truncate calyx.

Sclerocarpus elongatus (Greenm.) Greenman & Thompson, comb. nov.

S. Schiedeanus var. *elongatus* Greenm. Proc. Am. Acad. **32**:309. 1897.

Herbaceus; caule tereti ramoso erecto vel adscendenti 1–1.5 m. alto striato sparse strigoso plus minusve purpurascenti basi lignescenti; foliis brevipetiolatis trinerviis inferioribus oppositis superioribus alternis anguste lanceolatis 2.5–13 cm. longis 0.3–1.5 cm. latis acuminatis acutis integris vel remote denticulatis basi in petiolum gradatim angustatis supra tuberculato-hispidis subtus paulo pallidioribus secundum nervos venasque hirsutis; inflorescentiis laxe paniculatis, pedunculis gracilibus 0.5–8 cm. longis strigosis; capitulis 6–8 mm. altis; involucris subcampanulatis circiter 5 mm. altis, squamis biseriatis oblongo-lanceolatis ovatis vel subobovatis extus strigoso-pubescentibus ciliatis leviter atratolineatis; flosculis liguliferis 5–8, ligulis oblongis 6–10 mm. longis flavis; floribus disci circiter 30; achæniis maturitate obliquis striatis glabris.

Specimens examined:

Mexico: State of Morelos, fields around Cuernavaca, altitude 1585 m., 31 October, 1896, *C. G. Pringle, 6606* (Mo. Bot. Gard. Herb.), CO-TYPE; valley, near Cuantla, altitude 1370 m., 28 October, 1900, *C. G. Pringle, 9061* (Mo. Bot. Gard. Herb.). State of Vera Cruz, Ojapa, 30 June, 1910, *C. R. Orcutt, 5156* (Mo. Bot. Gard. Herb.).

Venezuela: without definite locality, *A. Fendler, 685* (Mo. Bot. Gard. Herb.).

A further study of co-type material of this species, supplemented by subsequent collections, and a careful comparison of it with *S. Schiedeanus* (DC.) Benth. & Hook. f., as represented by Schiede's number 225 preserved in the herbarium of the Missouri Botanical Garden and Pringle's number 8338 from the type locality, shows several important differences between the species and the plant referred to it as variety *elongatus*. The latter has narrowly lanceolate leaves, a much-branched stem, open inflorescence, and more numerous and smaller heads which altogether indicate that the plant in question should be regarded as of equal specific rank rather than a variety of *S. Schiedeanus*, hence it is here raised to specific rank and a somewhat amplified description is appended.

Flaveria longifolia Gray, Pl. Fendl. 88. 1849.

Var. **subtomentosa** Greenman & Thompson, var. nov.

Formæ typicæ habitu simili; caule plus minusve tomentoso; foliis lanceolato-attenuatis basi plerumque ampliatis amplexicaulibusque utrinque subtomentosis.

Specimens examined:

Mexico: State of San Luis Potosi, Minas de San Rafael, November, 1910, *C. A. Purpus, 4776* (Mo. Bot. Gard. Herb.), TYPE; Rio Verde, 17 November, 1910, *C. R. Orcutt, 5421* (Mo. Bot. Gard. Herb.); Rio Verde, 2–8 June, 1904, *Dr. Edward Palmer, 26* (Mo. Bot. Gard. Herb.).

Explanation of Plate

PLATE 24

Sisyrinchium angustissimum (Rob. & Greenm.) Greenm. & Thomp.

Mexico

From the type number, Pringle No. 4703, in the Herbarium of the Missouri Botanical Garden.

GREENMAN AND THOMPSON—DIAGNOSES OF FLOWERING PLANTS

COCKAYNE, BOSTON.

Explanation of Plate

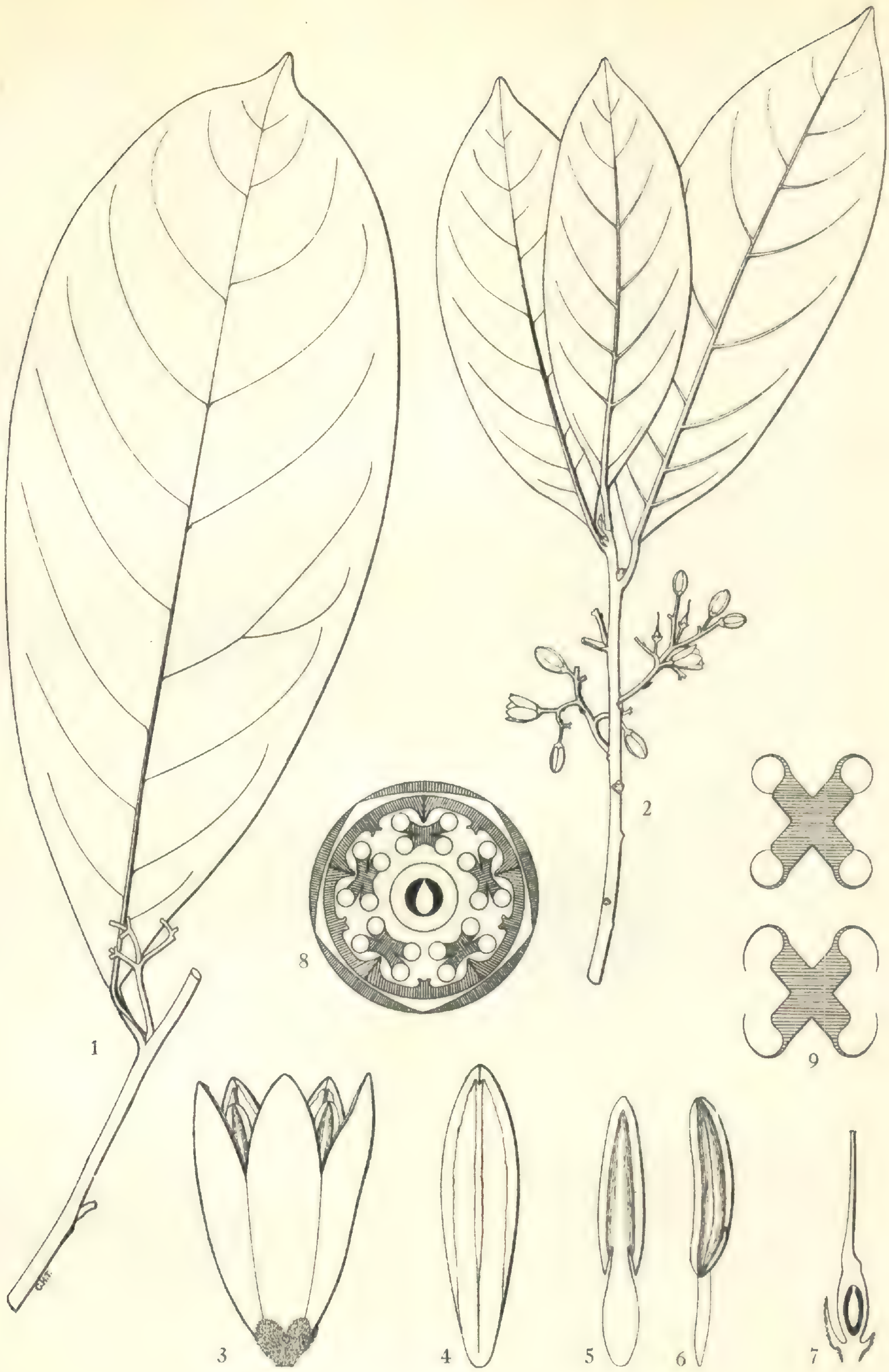
PLATE 25

Oecopetalum mexicanum Greenm. & Thomp.

Figs. 1 and 2, flowering branches; 3, flower; 4, inner face of petal; 5 and 6, front and side view of stamen; 7, longitudinal section of pistil; 8, diagrammatic cross-section of flower bud; 9, diagrammatic cross-section of anther before and after dehiscence.

Mexico

From the type specimen, Purpus No. 6159, in the Herbarium of the Missouri Botanical Garden.



GREENMAN AND THOMPSON—DIAGNOSES OF FLOWERING PLANTS

COCKAYNE, BOSTON.

Explanation of Plate

PLATE 26

Randia truncata Greenm. & Thomp.

Mexico

From the type specimen, Gaumer No. 713, in the Herbarium of the Missouri Botanical Garden.

GREENMAN AND THOMPSON—DIAGNOSES OF FLOWERING PLANTS

COCKAYNE, BOSTON.

ENZYME ACTION IN FUCUS VESICULOSUS L.

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Little is known regarding the metabolism of the *Fucaceæ*. The chemical nature of the chief accumulation products has not yet been sufficiently investigated. In fact, prior to 1905 very little work of importance had been contributed on the products of any group of the marine algæ. Even the chemical determination of the carbohydrates, for example, in some of the larger groups of algæ, afforded no suggestion as to the nature of these products. More activity in this general field of work has been manifest, however, since the date referred to. Diverse views prevailed regarding the nature of the various granules which had been long detected microscopically. In the earlier literature Hansteen's ('92, '00) opinion has generally dominated, by which it was claimed that the granular bodies of the cell—and particularly the larger vesicular forms—contain fucosan, a carbohydrate, which was considered the first visible product of photosynthesis. On the other hand, Crato ('92, '93) maintained, from microchemical reactions, that the larger vesicles, physodes as he called them, contained phloroglucin, or some derivative of this body. Müther and Tollens ('04) found a methylpentosan in *Fucus* and *Laminaria*, while Koenig and Bettels ('05) among others found glucose and fructose, as well as pentoses and methyl pentoses, in *Laminaria* after hydrolysis. Swartz ('11) gives an extensive summary of the previous work on carbohydrate occurrence in the algæ, and contributes much data on the digestion of the hemicelluloses, but she studied no brown algæ. The existence of reducing sugars in *Fucaceæ* was clearly shown by Tihomirow ('10). Recently the carbohydrates have been more completely investigated by Kylin ('12, '13). Nevertheless, much remains to be done on these products, while

the proteins (aside from agar agar and related compounds) and other organic substances are scarcely known.

In view of the very considerable data on the carbohydrate metabolism in higher plants, it seems particularly desirable to investigate further this relation in the brown algæ. Moreover, no general study having been made, as far as we could learn, of the enzymes of the *Fucaceæ*, it seemed possible that a determination of the more characteristic enzymes, and of their distribution in *Fucus*, might lead to a better comprehension of the nature of the metabolism of these plants. Accordingly, during the summers of 1913–14 we have made an examination of *Fucus vesiculosus* with respect to its enzyme content.

In preparing the Fucus material for study we have followed several of the customary methods which have been found satisfactory in yielding enzymes of a high degree of efficiency. Since our results with *Fucus* have been so generally negative with respect to the presence of the commoner enzymes of plant metabolism, it may be well to indicate briefly how the material was handled. The Fucus plants were obtained in quantity, apparently in a condition of active growth, and the material was carefully picked over to avoid the contamination of attached animals and smaller algæ, then washed, and finally treated by one of several methods. Some of it was hung in a shaded, warm room until quite dry and brittle, then ground in a mill to an extremely fine powder, the latter being preserved in dry bottles for extraction, as subsequently indicated. For other phases of the work the plants fresh from the water were ground almost to a pulp in a meat grinder, sometimes passing the material twice or oftener through the machine. In some cases this fresh pulp, further comminuted in a mortar, or an extract from it, was used directly, while in other cases an alcohol-acetone dry preparation was made from it—the latter by treating alternately with 95 per cent alcohol (15 minutes) and acetone (5–10 minutes) until practically dehydrated, with a final brief treatment with absolute alcohol or ether, when the material was spread out on filter paper to dry. The alcohol-acetone material was thoroughly pulverized in a mortar for further use.

In the preparation of extracts the dry material was treated with distilled water (usually 10 parts of water to 1 part of

material), or in some cases with sea-water, using commonly 20 per cent alcohol or 2–3 per cent toluene as a preservative. In general, toluene has proved the most satisfactory antiseptic. The filtered extract was then precipitated with 95 per cent alcohol, the precipitate caught on a filter, washed with alcohol and dried. In a few cases the extract was used direct, and in certain respects the common practices were variously modified in the hope of detecting some simple explanation of the large number of negative results.

The hydrolytic experiments were carried out in small Erlenmeyer flasks or test-tubes, and always in duplicate or triplicate. In addition, nearly every series was repeated once or oftener. A special effort was made to determine the presence of carbohydrases, and for this purpose weak solutions, usually 0.5 per cent, of starch, glycogen, dextrin, saccharose, maltose, and lactose were employed in numerous tests. No reduction, or no change in the reducing value of the substrate, by the Fehling method, was found in any case in our final experiments, although in some cases a relatively large quantity of the supposedly enzyme-containing material was used. We found it necessary to purify the best dextrin obtainable by precipitation with 95 per cent alcohol from a strong aqueous solution. In the preliminary experiments, and chiefly with one preparation, traces of reduction were found with glycogen, but in many later experiments this finding was not confirmed.

Owing to the consistently negative results with these carbohydrates it seemed possible that there might be an adjustment of enzyme action in *Fucus* such that a relation of the mineral salts, as in sea water, might be requisite for highest action. Consequently the enzyme solution in one large series of experiments was diluted with double strength sea-water; in another case the material was extracted with sea-water; and finally, fresh material was used, making with it a diffusion in sea water. In every instance the result was negative.

Another possibility then suggested itself, namely, that the presence of certain inhibiting substances might account for the absence of hydrolytic change. Accordingly, the effect of the Fucus material on the activity of taka diastase was determined in this way: To 10 grams of ground fresh material 100 cc. of

water and 1 gram of taka diastase were added, this being permitted to stand for 5 hours, as in extraction, and the filtrate from this extraction was tested upon starch solution. The results were positive, indicating that no free substances were present which could inhibit diastase action. In another test 1000 grams of Fucus material were divided into two lots of 500 grams each. To one of these, 5 grams of commercial malt diastase were added, and both were then treated by the alcohol-acetone method, and subsequently extracted and precipitated in the usual way. The material to which diastase had been added gave positive tests for the hydrolysis of carbohydrates in an extensive series with dextrin, glycogen, saccharose, and laminarin; but a solution of the precipitate from the lot receiving no diastase produced no changes in these substrates. These experiments included controls of several kinds. With every substrate, boiled material was also used, and it is interesting to note that the "enzyme" material increased in reducing power with boiling.

The tests referred to in the previous paragraph seemed all the more important inasmuch as the Fucus material had been found to be strongly acid, and it seemed possible that this acidity alone might prove an injurious factor. From the experiments just mentioned it is seen, however, that acidity could scarcely have been an important consideration. A quantitative determination of the acidity was nevertheless made, by titration with NaOH, and it was found to be about .0565 N HCl. There is a slight increase in the acidity, if the pulp is permitted to remain in water 12 hours.

Owing to the determination by many, as, for example, Müther and Tollens ('04), Kylin ('13), Swartz ('11), and others of the presence of hemicelluloses, especially pentosans, in the marine algæ, and, further, since the commoner carbohydrate enzymes had not been identified by us, it seemed desirable to examine the material for pentosanase. The most available pentosan was that of cherry gum, accordingly this material in fresh condition was obtained and utilized in many tests with the Fucus preparation, the flasks being maintained at temperatures ranging from 27–40° C. Although the experiments were permitted to run for a period of several days, no reduction above the amount found

in the controls was obtained, and certainly no pentosanase active on this material could be assumed to occur abundantly in Fucus tissues.

Only one series of tests has been made to identify cellulase in the material here reported upon, and the results are presented with much reserve. Precipitated cellulose, prepared from filter paper, was employed, and the experiments were conducted at 40° C. The indications were that slight cellulase activity may occur.

By means of the action of the alcohol-acetone preparation upon a 4 per cent olive oil-casein emulsion, the lipolytic activity was investigated in the usual way. With the emulsion used alcohol is most serviceable as a preservative. In the tests referred to there was no indication of hydrolysis after one week; so the preparations were permitted to stand for two months, but still without change. That the conditions in the above case were otherwise favorable for lipolytic action is shown by the fact that the same substrate yielded with an alga of another family a decidedly positive test in two days. Several series of experiments were likewise carried out for the determination of esterases. With methyl acetate, ethyl acetate, and ethyl butyrate the Fucus material produced no change, irrespective of the concentrations employed.

In some of our preliminary experiments it had appeared that urease was present, but a careful investigation of this point demonstrated an error in the earlier results, and no amidases were discovered through the action upon 0.5 per cent solutions of urea, acetamid, methylamine, asparagin, diphenylamine, and acetanilid. In these experiments NH_3 determinations were made according to the method of Folin.

No liquefaction of gelatin or of agar occurred during a ten-day interval in a large series of test-tubes arranged with these two substrates. In the different tests these media were made neutral, alkaline, and slightly acid. In the neutral and slightly acid tubes no observable change occurred; but in those tubes containing a higher percentage of acid — both in those containing the Fucus preparation and in the controls — general liquefaction occurred. It is obvious, therefore, that these gel-forming proteins are not noticeably affected by any enzymes

occurring in the Fucus material. More extensive series of tests were arranged to determine the presence of proteinases which might act upon some more widely distributed native proteins, such as albumin, casein, and legumin. No tests were made to determine the transformation of these bodies into proteoses or peptones, but the formaldehyde method of determining amino acids was employed, and in no case had any transformation of these substances proceeded to the amino acid stage.

Glucose, levulose, and galactose were used in two series of experiments designed to determine the presence of zymase in the alcohol-acetone Fucus powder. No sufficient evidence, however, of the occurrence of this enzyme was obtained even when the most delicate tests were employed to determine the liberation of CO_2. The action of Fucus extract from the alcohol-acetone preparation upon tannin was tested by means of quadruplicate experiments. Two concentrations of tannin were used, 1 per cent and 2.5 per cent. The determinations were made by means of Jean's iodine method, but in no case did the flasks receiving the Fucus extract exhibit hydrolysis greater than that shown by the controls. Neither prepared nor fresh Fucus material gave sufficient evidence of oxidase or peroxidase action to be considered positive. Negative results were obtained both by the direct method with gum guaiacum, and by the indirect method, in which the reagent mentioned is used with hydrogen peroxide, and apparently acidity is not a determining factor. The use of benzidine seemed to indicate oxidase activity, but it has been clearly shown that the ease with which this reagent undergoes "spontaneous" oxidation in boiled solutions necessitates caution in using it as a test of oxidase activity. Tests for catalase by the usual method, evolution of oxygen on the addition of hydrogen peroxide, have clearly indicated that this enzyme is widespread in *Fucus*. It should be noted that the findings with respect to oxidase and catalase activity are in agreement with those of Atkins ('14). Catalase was very generally identified by him in the algæ, but evidence of oxidase in the *Fucaceæ* was obtained only with benzidine as a reagent.

The unexpectedly negative character of the experimental work here briefly outlined prompted us to make many repeti-

tions of experiments and minor modifications in technique not referred to in this preliminary account. The nature of the results, furthermore, made it seem desirable that a much more general study be made of the abundance and distribution of the enzymes in the various families of the marine algæ, and such an investigation is now in progress by one of us.

It would seem idle to attempt here an explanation of the negative results obtained, yet two or three possibilities have occurred to us which may be mentioned. The conditions of life of the *Fucaceæ*, especially the temperature relation, make it possible to suspect that metabolic changes occur at a very slow rate. If this is the case, it might be assumed that the commoner metabolic enzymes might be present in such small quantity that an indication of their presence would not be apparent by utilizing the methods ordinarily employed. The very fact that the capacity for food accumulation, that is to say, the "storage" of food materials, has not become highly developed in these forms suggests that the usual enzymes might not be found in abundance. Nevertheless, if such is the case, it may be pointed out that the present methods of enzyme work are very inadequate when applied to metabolic processes in general dealing with the transformation of products which do not accumulate in some quantity in the cell. In this connection attention may be drawn to Arber's ('01) observation on the slow rate of transformation of starch in the thallus of *Ulva latissima*, where a darkening period of from three to five weeks was required for the disappearance of this product.

The other possibility which has suggested itself is that in the cells of the *Fucaceæ* there may occur inhibiting substances which upon the death of the cell may form with the enzymes compounds from which the ferments cannot be again recovered. We have no evidence of the existence of any such bodies. Further investigation of *Fucus* and related algæ should perhaps throw some light upon the negative evidence produced by our extensive data.

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New scientific names of plants and the final members of new combinations are printed in **bold face** type; synonyms and page numbers having reference to figures or plates, in *italic;* and previously published scientific names and all other matter, in ordinary type.

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